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ON SOURCES OF VITAMIN C

II. ALBERTA NATIVE FRUITS[1]

BY J. TUBA[2], G. HUNTER[3], AND L. L. KENNEDY[4]

Abstract

This paper is a survey of the ascorbic acid content of native Alberta fruits. Some wild fruits that are not usually eaten have been included in the investigation. The values of vitamin C for the most widely occurring and most generally used wild fruits are: blueberries, 5 to 18 mg.%; chokecherries, 5 to 19 mg. %; high-bush cranberries, 8 to 51 mg. %; pin cherries, 8 to 34 mg. %; raspberries, 14 to 40 mg. %; and saskatoon berries, 5 to 38 mg. %. These afford a considerable source of ascorbic acid.

In an addendum there are given the ascorbic acid values in miscellaneous plant materials.

Introduction

There is a great deal of information available on the vitamin C content of cultivated fruits in various countries but less attention has been given to fruits that grow in the wild or native state. An investigation by Waagen and Pett (8) dealing with the adequacy of vitamin C in Alberta diets has included analyses of fruits commonly used in Western Canada but only of cultivated varieties. During the past few years various European countries have attempted to achieve self-sufficiency in the matter of vitamin C in the diet, and as a result they have explored and developed native sources of supply. Workers in several of these countries, including Pyke and Melville (6) in Britain, have paid much attention to the ascorbic acid content of rose hips. In addition to this source, Iwanoff and Bukin (3) report studies in the U.S.S.R. on the vitamin C in germinating seeds, pine needles, native fruits, jams from native berries, and green walnuts.

We have already reported on the ascorbic acid content of evergreens and hips of Western Canadian species of roses (2, 7). The decrease in imports of citrus fruits and the urgency of the war suggested further the investigation of vitamin C in native Alberta fruits.

Experimental

Throughout the summer of 1943, the wild fruits were picked, generally at maturity, and were assayed for vitamin C frequently the same day or as

[1] *Manuscript received December 17, 1943.*
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soon after as possible. Material awaiting assay was kept in a refrigerator. Samples were obtained from a few other parts of the province, although not from as many as had originally been planned. When it was possible several different batches of each fruit were picked around Edmonton, usually from different locations, in order to obtain representative assays.

The fruit was extracted with metaphosphoric–hydrochloric-acid mixture, and aliquots were titrated with 2,6-dichlorophenolindophenol as described previously (7). For most assays, the fruit, of uniform ripeness, was crushed in a mortar. When crushing fruits with stones, such as chokecherries, a Waring blendor was used. For such fruits, in an additional sample, the flesh was separated from the stones by hand, weighed, and the percentage of pulp was then calculated. Thus the vitamin was estimated in the flesh alone. For certain fruits that gave deeply coloured extracts a modified form of the Graham and McHenry (4) method of titration was used.

Results

The ascorbic acid content of the hips of native Alberta roses varies from 1300 to 3500 mg. % in the wet flesh (7). Orange juice, one of the best sources from a cultivated fruit, contains, according to Pett and Cantor (5) from 10 to 60 mg. % and averages about 50 mg. %. The highest values recorded in fruit by Harris and Olliver (1) are 79 to 344 mg. % for cultivated black currants. The vitamin C levels in none of the fruits listed in Table I approach those found in rose hips, but they do compare very favourably with higher values recorded for cultivated fruits.

TABLE I

VITAMIN C IN ALBERTA NATIVE FRUITS

Name of fruit	Date picked (1943)	Source	Degree of maturity	No. of determinations	Vitamin C in mg. %	
					Range	Average
Amelanchier alnifolia (Saskatoon berries)	July 20 – 30	Edmonton	Under-ripe	5	13–38	27
	July 20 – 30	Edmonton	Ripe	6	15–30	24
	Aug. 10	Fort Saskatchewan	Ripe	2	15	15
	Aug. 10	Beaverlodge	Very ripe	2	5–7	6
	Aug. 13	Lake Wabamun	Ripe	2	7–11	9
	Aug. 18	Athabasca	Ripe	3	14–15	15
Crataegus species (Hawthorn fruit)	Aug. 26 – 31	Edmonton	Ripe	4	57–64	60
	Oct. 28	Olds	Ripe (dehydrated)	1	11	11
Fragaria americana (Strawberries)	July 16	Edmonton	Ripe	1	34	34
Fragaria virginiana (Strawberries)	July 16	Edmonton	Ripe	1	33	33
	Aug. 13	Lake Wabamun	Ripe	1	56	56
Grossularia setosa (Gooseberries)	June 28 – July 13	Edmonton	Green	15	21–123	60
	July 3 – 9	Edmonton	Ripe (red)	2	40–63	52
	July 31	Elk Island Park	Green	1	29	29

TABLE I—*Concluded*

VITAMIN C IN ALBERTA NATIVE FRUITS—*Concluded*

Name of fruit	Date picked (1943)	Source	Degree of maturity	No. of determinations	Vitamin C in mg. %	
					Range	Average
*Prunus melanocarpa** (Chokecherry)	Aug. 21 – Sept. 3	Edmonton	Ripe	5	7–11	10
	Aug. 26	Edmonton	Very ripe	1	5	5
	Sept. 9	Olds	Ripe	1	10	10
	Sept. 9	Olds	Ripe (sweet)	1	19	19
*Prunus pennsylvanica** (Pin cherries)	July 30 – Aug. 9	Edmonton	Ripe	2	25–34	30
	Aug. 10 – 26	Edmonton	Very ripe	3	8–10	9
Ribes species (White currants)	Aug. 10	Olds	Ripe	2	53	53
Ribes species (Black currants)	Aug. 9	Beaverlodge	Ripe	3	94–170	134
	Aug. 12	Olds	Ripe	2	88–90	89
Ribes americanum (Black currants)	Aug. 23 – 30	Edmonton	Ripe	4	56–134	89
Ribes Hudsonianum (Black currants)	Aug. 30	Edmonton	Ripe	2	38–44	41
Ribes glandulosum (Red currants)	July 12 – 15	Edmonton	Ripe	6	30–80	55
Ribes triste (Red currants)	July 31	Elk Island Park	Ripe	1	50	50
	Aug. 10	Olds	Ripe	2	52–53	53
Rubus chamaemorus (Baked apple berries or cloud berries)	Aug. 18	Athabasca	Ripe	2	98–114	106
Rubus idaeus (Raspberries)	July 30 – Aug. 11	Edmonton	Ripe	6	14–40	27
	Aug. 13	Lake Wabamun	Ripe	2	35–39	37
	Aug. 21	Athabasca	Ripe	2	32–34	33
Rubus pubescens (Dewberries)	July 23	Edmonton	Ripe	1	20	20
	July 31	Elk Island Park	Ripe	1	14	14
	Aug. 13	Lake Wabamun	Ripe	1	28	28
Vaccinium caespitosum (Blueberries)	Aug. 12	Olds	Ripe	2	11–18	15
Vaccinium canadense (Blueberries)	Aug. 13	Lake Wabamun	Ripe	2	7	7
	Aug. 18	Athabasca	Ripe	2	5–7	6
	Sept. 22	Waterways	Ripe	1	13	13
Vaccinium vitis-idaea (Bog cranberries)	Aug. 2	Winterburn	Under-ripe	1	16	16
	Aug. 18	Nestow	Ripe	2	16	16
	Sept. 9	Athabasca	Ripe	1	15	15
*Viburnum pauciflorum** (High-bush cranberries)	Aug. 13	Lake Wabamun	Ripe	2	8	8
	Aug. 18	Athabasca	Ripe	2	9–10	10
*Viburnum trilobum** (High-bush cranberries)	Aug. 21 – Sept. 3	Edmonton	Under-ripe	2	22–51	36
	Aug. 26	Edmonton	Ripe	1	11	11
	Sept. 9	Olds	Ripe	1	15	15
	Oct. 12	Beaverlodge	Ripe	1	9	9
	Oct. 28	Olds	Ripe	1	14	14

* *Values per 100 gm. pulp.*

It is interesting to compare the vitamin C content of native fruits with that of corresponding cultivated fruits as reported by Harris and Olliver (1). For some fruits the comparative ascorbic acid levels are (cultivated fruit values in parentheses): black currants, 88 (231); red currants, 53 (46); gooseberries, 47 (40); haws, 60 (92); strawberries, 41 (61).

It should be noted that an advanced degree of maturity causes a decrease in vitamin C in some fruits. This is quite evident in chokecherries, high-bush cranberries, pin cherries, and saskatoons.

The "baked apple berries" or cloud berries are used extensively in the Scandinavian countries for making jam, but they are quite scarce in Alberta.

Some of the most widely distributed and commonly used of native fruits, while not of high ascorbic acid content, are, nevertheless, important sources of the vitamin. The average values of vitamin C for some of these from Table I are; blueberries, 10 mg. %; chokecherries, 11 mg. %; high-bush cranberries, 15 mg. %; pin cherries, 20 mg. %; raspberries, 32 mg. %; and saskatoon berries, 16 mg. %.

The native fruits included in Table II are not commonly eaten. One, the baneberry, is listed among toxic wild fruits, although feeding experiments with rats did not show any deleterious effects.

TABLE II

VITAMIN C IN WILD FRUITS NOT GENERALLY USED

Name	Date picked (1943)	Source	Degree of maturity	No. of determinations	Vitamin C in mg. %	
					Range	Average
Actaea alba (Baneberry)	July 22	Edmonton	Ripe	1	101	101
Aralia nudicaulis (Sarsaparilla)	Aug 16	Edmonton	Ripe	2	17–22	20
Cornus canadensis (Bunchberries)	Aug. 13	Lake Wabamun	Ripe	2	25–26	26
	Aug. 18	Athabasca	Ripe	2	32–39	36
Cornus stolonifera (Dogwood berries)	Aug. 15	Edmonton	Under-ripe	2	86–91	89
	Sept. 15	Edmonton	Ripe	1	112	112
*Disporum trachycarpum** (Wild orange berries)	Aug. 9	Edmonton	Ripe	1	37	37
	Sept. 7	Edmonton	Ripe	2	42–55	49
Symphoricarpos albus (Buck brush berries)	Sept. 23 - Oct. 18	Edmonton	Ripe	3	58–63	61

* *Estimations were made on flesh alone.*

Addendum

During the course of the investigations recorded in the foregoing part of this paper, assays were made on plant materials that could not be included therein, but that are of some interest in the matter of their vitamin C content.

Caragana, which is widespread in Western Canada, contains relatively large amounts of the vitamin in the leaves. Some common wild greens such as the dandelion, lamb's-quarters, and nettles have been examined. Moss does not appear to be a potential winter source. The new needles of the spruce contain markedly smaller amounts of ascorbic acid than the older ones.

TABLE III

THE VITAMIN C CONTENT OF MISCELLANEOUS PLANT MATERIALS

Name of plant	Date (1943)	Material assayed	No. of determinations	Vitamin C in mg. %	
				Range	Average
Caragana arborescens (Caragana)	June 11 – Sept. 16	Leaves	9	291–575	421
	July 27	Seeds (green)	1	101	101
	July 27	Pods (green)	1	29	29
Chenopodium album (Lamb's-quarters)	June 3 – Sept. 22	Leaves	3	120–218	157
Corylus cornuta (Hazelnuts) (green)	July 21	Endosperm	1	7	7
	July 21	Shell	1	8	8
	July 21	Husk	1	42	42
Ledum groenlandicum (Labrador tea)	Aug. 5	Leaves	2	265–305	285
Polytrichum commune (Moss)	Oct. 26		1	Negligible	
Petroselinum hortense (Parsley)	Sept. 17 – 22	Leaves	2	359–423	391
Picea alba (Spruce)	Jan. 14	Bark	2	119–138	128
	June 2	New needles	2	46–47	47
	June 2 – 22	Old needles	10	90–345	179
Populus tremuloides (White poplar)	Jan. 14	Bark	2	99–112	106
Taraxacum officinale (Dandelion)	May 26	Leaves	2	75–85	80
	May 26	Flowers and green calyx	1	53	53
	May 26	Roots	1	Negligible	
Urtica gracilis (Nettles)	Aug. 17	Leaves	1	239	239

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RESEARCHES ON DROUGHT RESISTANCE IN SPRING WHEAT

III. SIZE AND FREQUENCY OF STOMATA IN VARIETIES OF *TRITICUM VULGARE* AND OTHER *TRITICUM* SPECIES[1]

By J. E. Birdsall[2] and K. W. Neatby[3]

Abstract

Significant differences between varieties and hybrid strains of *Triticum vulgare* with respect to the length and frequency of stomata per unit area are established. In studies involving 12 different species and four different chromosome numbers, it is demonstrated that increasing chromosome number is associated with larger and fewer stomata. Both between species and between varieties within species, a negative relation between size and frequency of stomata is demonstrated. Data on stomata were studied in relation to yield of grain under field conditions by means of the correlation coefficient. Significant relations are established and it is concluded that stomatal determinations may be of use in selecting for high yield.

Introduction

In cereal crops, yield of grain under field conditions is influenced by a multiplicity of genetical and environmental factors. In order to classify a single genotype according to its yielding capacity over a given territory, it is necessary to 'sample' the environment in time and space by extending yield trials over several years and under varying soil and climatic conditions. Furthermore, the importance of local adaptations is emphasized by the occasionally pronounced interaction between varieties and districts. Then, since the inheritance of yielding capacity is undoubtedly complex, large populations of hybrid material must be grown if the yield of the highest yielding parent is to be recovered or improved upon. To make matters worse, especially from the point of view of drought resistance, the experimental error involved in field trials is usually greater in dry years or dry districts than under more humid conditions.

During the years 1935 to 1939, inclusive, at the University of Alberta, researches on drought resistance were directed at the identification of more or less readily recognizable characters, associated with yield under field conditions in dry areas, that might aid in selecting for high yield under conditions of limited moisture supply. Since the number of strains that can be subjected to field tests for yield is necessarily limited, the recognition of any character or characters by means of which low yielding material could be eliminated in early hybrid generations would facilitate raising the general level of yield and so enhance the likelihood of isolating valuable strains in yield tests.

It is unnecessary to describe in detail the negative results obtained. Resistance of varieties and hybrid strains to atmospheric drought has been tested in

[1] *Manuscript received November 13, 1943.*

Contribution from the Department of Field Crops, University of Alberta, Edmonton, Alta., with financial assistance from the National Research Council of Canada.

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a machine especially constructed for the purpose(4, 5, 9); but the experimental error was very great and no relation with yield under field conditions was established.

The tillering capacity of some hundreds of varieties and strains has been determined; but notwithstanding the establishment of varietal differences in individual years, the interaction between years and varieties was so great that this character could have little, if any, selection value.

The comparative survival of seedlings of 22 varieties of wheat grown with the roots immersed in sugar solutions with and without mineral nutrients was studied intensively (9). Statistically significant differences between varieties within individual tests were established; but the interaction between varieties and tests was such that any relation with yield under field conditions was out of the question.

The total length of main roots of several series of varieties and hybrid strains was determined on plants grown for one month in containers designed and constructed for that purpose. Varietal differences were established, but there was no clear relation with yield under field conditions, and the tests were almost as expensive and time-consuming as regular yield trials.

This report is concerned with variations in the size and frequency of stomata per unit area, and the possible utility of these variations in selecting for high yield.

Material and Methods

All stomatal determinations were made on the varieties and hybrid strains that were used in yield tests being conducted in connection with the program of breeding wheat for drought resistance, except those involving the study of stomata in relation to chromosome numbers. The material in the yield tests consisted of standard varieties, introductions, and intervarietal hybrids. Most of the introductions were of Russian origin. Studies were also made on different varieties and species of *Triticum*, representing 12 species and four chromosome groups, grown in small observation plots. Approximately 260,000 stomata in 8000 microscope fields were counted and the length of 16,000 was determined.

For making counts, a No. 8 objective and a No. 15 ocular were used and for measuring stomata, an 8× objective and an ocular screw-micrometer. For counts made in 1937, upper leaf surfaces were used, but all subsequent counts and measurements were made on lower leaf surfaces. All determinations were made at approximately the same distance from the base of the leaf blade, the distance being determined by placing the base at the left-hand edge of the microscope stage. Leaves were held on an 8 × 4 cm. slide by means of a piece of cardboard of the same dimensions, glued to the slide at one edge. A hole, 1 cm. in diameter, was cut in the centre of the cardboard in order to expose a portion of the leaf. Measurements were made on closed stomata, closure being insured by placing the detached leaves in darkness for one hour. Ten stomata were measured on each of five leaves of each

variety, lengths of entire stomata (not apertures) being determined. In order to avoid or minimize possible error due to inter-row variation in size on individual leaves, one stoma in each of 10 rows on each leaf was measured. For determining frequencies all stomata in one microscope field on each of 10 leaves of each variety were counted. At the periphery of the field, if one-half or more of a stoma was in view, it was counted. Determinations on the terminal leaves were made when the plants had just headed and those on seedling leaves when the plants were in the three-leaf stage.

Intervarietal Differences in the Length and Frequency of Stomata

Miller (6) classified a large number of species and varieties of crop plants on the basis of stomatal characteristics and concluded that the number of stomata per unit area of leaf surface is, within limits, specific for varieties and species. An inverse relation between frequency and size of stomata has been demonstrated by Eckerson (2) and Muenscher (7).

The data on length of stomata, derived from material in yield trials, are summarized in Table I, and the statistical analyses are recorded in Table II. It will be observed that two of the tests involved the use of seedling leaves, and the other three of terminal leaves. The highly significant variance derived from differences between test means is almost entirely due to the fact that seedling leaves have much longer stomata than do terminal leaves. The mean difference between the two independent 1939 tests is entirely insignificant. The significance of varietal differences within individual tests cannot be determined because interplot variability was not sampled. It can be asserted with confidence, however, that interplot variability was relatively unimportant, since differences between test means for terminal or seedling leaves were comparatively small even though different years were involved. Varietal differences are established beyond a doubt.

TABLE I

LENGTHS OF STOMATA, IN MICRONS, OF 52 VARIETIES AND HYBRIDS OF *Triticum vulgare*

Variety	Seedling leaves		Terminal leaves			Means		
	1939	1938	1938	1939	1939	5-Test	4-Test	2-Test
Marquis	74.8	72.2	60.6	54.8	55.9	63.7	60.9	55.4
Caesium	68.5	64.6	56.9	53.1	53.1	59.2	56.9	53.1
Caesium × Marquis	72.4	68.6	58.6	51.8	55.5	61.4	58.6	53.6
	68.6	68.9	58.5	56.3	54.4	61.3	59.5	55.4
	73.5	69.9	57.9	56.6	55.6	62.7	60.0	56.1
	72.9	71.4	60.2	56.2	57.1	63.6	61.2	56.6
	76.2	68.7	59.7	54.7	55.0	62.9	59.4	54.8
	72.6	72.4	61.3	58.5	57.1	64.4	62.3	57.8
	75.3	68.7	57.7	55.6	57.3	62.9	59.8	56.4
	74.3	70.0	59.3	57.1	57.8	63.7	61.0	57.4
	71.3	68.3	59.6	55.5	55.2	62.0	59.6	55.4

TABLE I—*Concluded*

LENGTHS OF STOMATA, IN MICRONS, OF 52 VARIETIES AND HYBRIDS OF *Triticum vulgare*—*Concluded*

Variety	Seedling leaves		Terminal leaves			Means		
	1939	1938	1938	1939	1939	5-Test	4-Test	2-Test
Caesium × Marquis—*Concluded*	77.0	72.1	61.3	54.3	57.5	64.4	61.3	55.9
	74.7	65.2	58.9	56.6	55.2	62.1	59.0	55.9
Double Cross × Milturum	72.7	73.1	60.2	55.7	55.3	63.4	61.1	55.0
	82.0	72.5	63.7	59.0	59.4	67.3	63.6	59.2
	78.9	74.0	66.2	60.5	60.9	68.1	65.4	60.2
	82.0	75.9	58.5	57.5	56.1	66.0	62.0	56.8
	76.0	75.0	58.6	55.6	57.1	64.5	61.6	56.4
	74.3	73.5	60.4	56.0	58.1	64.5	62.0	57.0
	72.3	69.5	60.2	56.3	55.9	62.8	60.5	56.1
	74.6	69.4	59.6	52.5	58.0	62.8	59.9	55.2
	77.9	75.5	59.0	56.1	56.5	65.0	61.8	56.3
	78.6	73.2	61.0	56.4	56.9	65.2	61.9	56.6
	76.6	76.3	59.0	55.9	54.9	64.5	61.5	55.4
	74.8	73.4	58.0	57.1	56.1	63.9	61.2	56.6
Apex		68.9	61.1	57.1	56.2		60.8	56.6
Canus		71.9	61.1	55.8	56.4		61.3	56.1
Double Cross		71.6	62.7	57.7	57.7		62.4	57.7
Milturum		66.5	57.9	54.6	57.6		59.2	56.1
Red Bobs		65.2	52.7	49.8	50.4		54.5	50.1
Thatcher		75.4	62.5	58.4	56.6		63.2	57.5
Lutescens				55.4	54.2			54.8
Regent				56.0	56.6			56.3
Caesium × Marquis				52.1	56.0			54.0
				57.3	55.2			56.2
				54.3	54.0			54.2
				53.6	55.2			54.4
				55.8	59.5			57.6
				57.4	55.9			56.6
				55.7	54.4			55.0
				58.3	55.9			57.1
				56.0	56.0			56.0
				53.3	54.4			53.8
				54.7	53.5			54.1
				52.9	53.5			53.2
Marquis × Caesium				51.1	53.4			52.2
Reward & Caesium				52.4	51.0			51.7
				53.8	52.7			53.2
				53.3	54.4			53.8
				52.4	53.6			53.0
				52.4	51.8			52.1
				55.2	55.2			55.2
Minimum significant difference						1.2	1.2	1.2

TABLE II

ANALYSES OF VARIANCE OF DATA IN TABLE I

	Degrees of freedom	Mean square	*F*	1% point
5-test means				
Varieties	24	17.69	4.78	1.99
Tests	4	1939.06	524.07	3.52
Varieties × tests	96	3.70		
4-test means				
Varieties	30	15.93	5.27	1.92
Tests	3	1543.64	511.14	4.01
Varieties × tests	90	3.02		
2-test means				
Varieties	51	7.34	4.99	1.94
Tests	1	2.68	1.82	7.16
Varieties × Tests	51	1.47		

Data on frequency of stomata and the statistical analyses thereof are recorded in Tables III and IV. The material was derived from the same sources as those used for the measurement of stomata, except that the 31 varieties used for counting stomata in 1937 were grown for making measurements in 1938. The statistical significance of varietal differences is less pronounced with frequencies than with length, though the magnitude of the differences is roughly of the same order. The mean difference between tests in the two-test analysis (Table IV) is significant, while with lengths it was not. This indicates that stomatal frequencies are more subject to environmental influences than are lengths.

TABLE III

FREQUENCY OF STOMATA, PER SQ. MM., ON 52 VARIETIES AND HYBRIDS OF *Triticum vulgare*

Variety	Seedling leaves		Terminal leaves			Means		
	1939	1938	1937	1939	1939	5-Test	4-Test	2-Test
Marquis	25.1	22.7	69.7	46.6	49.6	42.7	47.2	48.6
Caesium	24.6	20.6	72.3	54.2	51.4	44.6	49.6	52.8
Caesium × Marquis	27.1	19.7	66.4	48.8	51.1	42.6	46.5	50.0
	29.0	19.7	70.4	50.7	47.1	43.4	47.0	48.9
	28.4	17.9	67.0	44.2	47.1	40.9	44.0	45.6
	26.7	19.7	66.0	40.3	47.4	40.0	43.4	43.8
	22.3	16.5	67.0	47.7	49.1	40.5	45.1	48.4
	28.1	20.0	71.0	47.8	47.0	42.8	46.4	47.4
	31.4	21.9	68.3	50.0	48.8	44.1	47.2	49.4
	27.1	22.7	79.0	45.7	47.8	44.5	48.8	46.8
	28.2	24.0	72.0	50.8	45.7	44.1	48.1	48.2
	27.8	23.6	69.0	49.6	43.0	42.6	46.3	46.3
	25.1	20.0	70.3	53.5	49.4	43.7	48.3	51.4

TABLE III—*Concluded*

FREQUENCY OF STOMATA, PER SQ. MM., ON 52 VARIETIES AND HYBRIDS OF *Triticum vulgare*—*Concluded*

Variety	Seedling leaves		Terminal leaves			Means		
	1939	1938	1937	1939	1939	5-Test	4-Test	2-Test
Double Cross × Milturum	29.8	22.4	68.2	49.8	48.6	43.8	47.2	49.2
	24.6	18.7	60.9	46.2	40.0	38.1	41.4	43.1
	23.7	20.3	61.6	42.7	49.3	39.5	43.5	46.0
	25.8	21.4	66.3	47.8	45.2	41.3	45.2	46.5
	25.7	21.9	66.6	46.0	47.8	41.6	45.6	46.9
	27.8	19.4	64.9	45.7	40.8	39.7	42.7	43.2
	28.0	21.0	62.3	47.4	43.0	40.3	43.4	45.2
	26.6	20.7	67.2	47.7	47.0	41.8	45.6	47.4
	25.3	20.9	66.9	50.7	43.2	41.4	45.4	47.0
	24.1	19.7	67.2	49.7	45.2	41.2	45.4	47.4
	23.8	23.1	71.7	47.3	44.3	42.0	46.6	45.8
	24.0	21.9	72.6	50.8	48.7	43.6	48.5	49.8
Apex		19.3	69.6	49.8	46.6		46.3	48.2
Canus		19.9	69.0	48.8	45.9		45.9	47.4
Double Cross		22.7	58.9	51.5	45.4		44.6	48.4
Milturum		21.4	95.3	49.0	54.4		55.0	51.7
Red Bobs		23.4	79.1	60.4	57.5		55.1	59.0
Thatcher		23.0	56.6	49.4	43.9		43.2	46.6
Lutescens				45.9	50.3			48.1
Regent				49.3	45.6			47.4
Caesium × Marquis				46.9	37.3			42.1
				49.8	45.6			47.7
				49.7	40.2			45.0
				49.7	46.0			47.8
				44.3	42.3			43.3
				45.7	50.6			48.1
				46.2	48.3			47.2
				42.2	43.4			42.8
				47.3	46.7			47.0
				52.7	47.6			50.2
				47.7	49.4			48.6
				53.2	48.8			51.0
Marquis × Caesium				49.3	43.4			46.4
Reward × Caesium				48.7	46.6			47.7
				53.4	54.5			54.0
				49.6	44.9			47.8
				51.7	55.0			53.4
				45.7	50.7			48.2
				49.3	51.0			50.2
Minimum significant difference						1.6	2.6	2.9

TABLE IV

ANALYSES OF VARIANCE OF DATA IN TABLE III

	Degrees of freedom	Mean square	*F*	1% point
5-test means				
Varieties	24	15.10	2.25	1.99
Tests	4	8981.00	1340.45	3.52
Varieties × tests	96	6.70		
4-test means				
Varieties	30	35.99	2.63	1.92
Tests	3	11935.91	873.15	4.01
Varieties × tests	90	13.67		
2-test means				
Varieties	51	17.99	2.20	1.94
Tests	1	60.62	7.43	7.16
Varieties × tests	51	8.16		

Interspecific Differences in the Length and Frequency of Stomata

Blakeslee (1) reports that artificially doubling chromosome numbers results in larger stomata. His conclusion is based on work with hemp where stomata in the floral bracts of female plants were measured.

Sax and Sax (11), with *Tradescantia* spp., and Franco (3), with *Coffea* spp., have demonstrated the existence of a relationship between the number of chromosomes and the size and frequency of stomata. The relations with size were positive and with frequency, negative. Sax (10), on the other hand, studied herbarium specimens of several genera of trees and shrubs, and observed that with some exceptions there was a positive correlation between number of chromosomes and number of stomata.

In 1939, a study of chromosome size and frequency was undertaken with 49 varieties including representatives of four different chromosome groups. Chromosome numbers were not actually determined but botanical characteristics and known interfertility relations leave little doubt as to the accuracy of the classification.

Terminal leaves were used and, as in other studies, 10 stomata were measured on each of 10 leaves of each variety and stomata in one microscope field were counted on 10 leaves of each variety.

The essential features of the results will be found in Table V, and the details are assembled in Table VI. It is apparent that, as chromosome numbers increase, the length of stomata increases and the frequency decreases. That stomatal length and frequency are not entirely controlled by chromosome numbers is evidenced by pronounced differences between varieties within chromosome groups. Indeed, the variability within chromosome groups is such that varieties could not be placed with certainty in the correct chromosome groups on the basis of stomatal data presented here. Milturum 0321, for example, would fall in the 28-chromosome group.

TABLE V

THE MEAN LENGTH AND FREQUENCY OF STOMATA IN DIFFERENT CHROMOSOME GROUPS OF THE GENUS *Triticum*

Chromosome number (2n)	Length, μ		Frequency, per sq. mm.	
	Number of varieties	Mean length	Number of varieties	Mean frequency
14	1	41.7	1	82.1
28	8	46.1	24	63.9
42	6	53.3	22	51.9
56	2	59.2	2	46.6

TABLE VI

MEAN STOMATAL FREQUENCIES AND LENGTHS FOR WHEAT VARIETIES INVOLVING 12 SPECIES AND FOUR CHROMOSOME GROUPS

Variety name	1939 Plot No.	Frequency per sq. mm.	Length, μ
14-chromosome group			
T. monococcum	1	82.1	41.7
28-chromosome group			
T. durum			
Velvet Don	21	61.6	
Melanopus	20	62.2	
Albidum 0604	23	65.9	
Russian 123	13	63.5	
Pentad	11	63.9	
Iumillo	12	65.7	
C.I. 7513	19	55.8	46.2
Pellisier	14	62.3	
Sigfusson	15	60.4	
Mindum	35	67.7	
Hordeiforme 010	34	74.0	43.7
Kubanka	33	65.2	
Mindum	30	66.6	
Hordeiforme 0189	28	62.9	
Mindum × Pentad	40	58.4	
Kahla 5529	39	61.9	
T. turgidum			
*D*371	331	74.4	40.3
Alaska	10	64.9	45.7
T. dicoccum			
Common Emmer	4	62.6	47.9
T. polonicum			
Polish	9	67.2	44.6
T. persicum	276	68.9	
	275	58.8	50.4
	453	58.2	
T. timopheevi	299	60.8	50.2
Mean		63.9	46.1

TABLE VI—*Concluded*

MEAN STOMATAL FREQUENCIES AND LENGTHS FOR WHEAT VARIETIES INVOLVING 12 SPECIES AND FOUR CHROMOSOME GROUPS—*Concluded*

Variety name	1939 Plot No.	Frequency per sq. mm.	Length, μ
42-chromosome group			
T. spelta			
Common Spelt	7	46.4	55.4
T. compactum			
Little Club	56	53.7	51.1
Hybrid 143	55	52.5	
T. vulgare			
Albidum 0721	87	58.1	
Dicklow	93	63.8	
Marquis	142	46.4	55.8
Garnet	127	52.7	
Red Bobs	135	60.6	50.0
D 381	342	52.0	
Kenya	472	52.3	
Double Cross	200	47.1	56.1
Milturum 0321	184	60.6	51.6
Baart	117	48.4	
Pilcraw	108	51.5	
Pacific Bluestem	107	51.5	
D 433	348	50.0	
C 518	436	49.0	
C 409	437	45.9	
8 *A*	439	49.4	
Type 14	440	45.3	
Type 13	441	52.5	
Gular	447	51.4	
Mean		51.9	53.3
56-chromosome group			
"Short"	315	44.6	62.3
"Tall"	316	48.6	56.1
Mean		46.6	59.2

TABLE VII

ANALYSES OF VARIANCE OF STOMATAL DATA FROM 49 VARIETIES INVOLVING FOUR DIFFERENT CHROMOSOME NUMBERS

	Degrees of freedom	Mean square	*F*	1% point
Length of stomata				
Chromosome groups	3	7019.6	131.5	4.08
Varieties	13	523.0	9.8	2.41
Between leaves (error)	68	53.4		
Within leaves	765	13.1		
Frequency of stomata				
Chromosome groups	3	8334.2	207.7	3.83
Varieties	45	224.6	5.6	1.58
Remainder	441	40.1		

The data were subjected to statistical analysis, the results of which are presented in Table VII. It is clear that the chromosome groups are the most important factor contributing to the total variability. It is necessary to point out, however, that provision for estimating soil variability was not made, since the data were obtained from single plots.

It is unlikely that soil variability contributed materially to observed differences; but its influence would certainly be greater between plots than within plots, and its neglect would inevitably increase the apparent significance of varietal differences.

Relation Between Length and Frequency of Stomata

A negative relation between length and frequency of stomata was apparent in the study of varieties and species grouped according to chromosome number. That this relation exists within *Triticum vulgare* is evidenced by the correlation coefficients in Table VIII.

TABLE VIII

RELATION BETWEEN LENGTH AND FREQUENCY OF STOMATA IN VARIETIES AND STRAINS OF *T. vulgare* AS MEASURED BY THE CORRELATION COEFFICIENT

Material	Number of tests	Year of determination		r
		Frequency	Length	
75 varieties, terminal leaves	1	1937	1938	−.55*
52 varieties, terminal leaves	2	1939	1939	−.42*
31 varieties, terminal leaves	3	1937	1938	−.54*
		1938	1939	
		1939	1939	
25 varieties, seedling leaves	1	1939	1938	−.34
25 varieties, seedling leaves	2	1938	1938	−.19
		1939	1939	

* *Exceeds the 1% point.*

The basis of this relation may not be exclusively genetical. Differences in size and vigour of plants have a bearing on stomatal characteristics in that small plants tend to have small stomata and a relatively large number per unit area. Whiteside (12) found that stomatal frequency in both Marquis and Canus wheat was almost doubled and stomatal size was greatly reduced by subjecting plants to soil drought. In correlations based on single tests, soil variability would inevitably influence the magnitude of coefficients. However, in the case of the coefficient based on 31 varieties and three different tests, since variety means were used (i.e. 30 degrees of freedom), environmental influences are practically negligible and the coefficient, $r = -.54$, may be accepted as an indication of genetic relationship.

Further evidence of the reality of genetical variation is forthcoming from a subdivision of the single test including 75 varieties ($r = -.55$). Forty-three

of these were Caesium × Marquis and Double Cross × Milturum strains and the remainder miscellaneous varieties. The correlation coefficient calculated on the latter group only was −.86.

In view of the convenience of using seedling material grown in the greenhouse, the reliability of determinations made on such material was investigated. Two series, one including 75 varieties and another consisting of 25 of the same varieties, were used. Counts and measurements of stomata were made on terminal leaves in the field and on seedling leaves in the greenhouse. Correlation coefficients calculated from the data obtained are recorded in Table IX. These show that independent determinations of length made on terminal leaves in the field were fairly closely related; but the relation was weaker when seedlings grown in the greenhouse were used. Seedling leaf determinations were apparently more subject to error than were those made on terminal leaves. Furthermore, independent frequency determinations were not as closely related as were independent measurements of length.

TABLE IX

CORRELATION COEFFICIENTS CALCULATED FROM STOMATAL DETERMINATIONS MADE ON TERMINAL LEAVES IN THE FIELD AND ON SEEDLING LEAVES IN THE GREENHOUSE

Material	Correlation coefficients	
	75 varieties	25 varieties
Length		
Terminal leaves with terminal		+.77*
Seedling leaves with seedling		+.51*
Seedling leaves with terminal	+.40*	
Frequency		
Terminal leaves with terminal		+.49*
Seedling leaves with seedling		+.26*
Seedling leaves with terminal	+.56*	

* *Exceeds the 1% point.*

Relation Between Yield and Stomatal Characteristics

Yield data were available from several different yield tests for 31 varieties and hybrid strains on which stomatal counts and measurements had been made. Of these, 14 were Caesium × Marquis strains, 12 were Double Cross × Milturum strains and five were miscellaneous varieties. Most of the yield tests consisted of four replicate plots of each variety and the remainder had three replicates.

The data are summarized in Table X. Yield appears to be positively related to stomatal frequency, while the relation with stomatal size is negative. Since all the material in the yield tests had been more or less rigorously selected for yield, the correlation coefficients are probably underestimates of the true relation. That is to say, since only the upper end of the yield distribution is

TABLE X

CORRELATION COEFFICIENTS CALCULATED FROM DATA ON STOMATA AND YIELD OF GRAIN

Mean yield	Stomatal characters	r
	Frequency	
5 tests	Terminal leaves, 1937	+.11
5 tests	Terminal leaves, means of three tests	+.39*
4 tests	Terminal leaves, means of three tests	+.09
6 tests	Terminal leaves, means of two tests	+.09
5 tests	Seedling leaves, 1938	+.04
	Length	
5 tests	Terminal leaves, 1938	−.36*
5 tests	Terminal leaves, means of three tests	−.40*
4 tests	Terminal leaves, means of three tests	−.32
6 tests	Terminal leaves, means of two tests	−.34
5 tests	Seedling leaves, 1938	−.54*

* *Exceeds the 5% point.*

sampled, high coefficients could not be expected even if the true relation were very close. The work should be repeated with unselected material and, until this is done, the possible utility of stomatal characters in selection for yield must remain an open question.

Relation Between Protein Content of Grain and Stomatal Characteristics

A genetical negative relation between yield and protein content of wheat has been demonstrated by Neatby and McCalla (8) and evidence of a negative relation between yield and stomatal length has been submitted above. In view of these facts, a positive relation between protein content and stomatal length is to be expected. That this relation actually does exist is indicated by the correlation coefficients in Table XI. The second coefficient in the table for length ($r = -.23$) does not conform to the general relation indicated by the others, and the second coefficient for frequency ($r = -.12$), though it does conform, is not statistically significant. This can be explained by the fact that the yield data were obtained in 1939 from two tests at Castor, Alta., and one at Scott, Sask. In all three tests the grain was badly shrivelled by excessive heat during ripening and this resulted in relatively high protein percentages. This was especially noticeable in the Caesium × Marquis strains. The negative relation between protein content and stomatal frequency indicated in Table XI is surprisingly strong as compared with the positive relation between frequency and yield (Table X). Somewhat higher values could be expected since protein content can be determined more accurately than can yield. However, if this were a complete explanation, larger correlation coefficients for protein content and length of stomata would be expected.

TABLE XI

CORRELATION COEFFICIENTS CALCULATED FROM DATA ON STOMATA AND PROTEIN CONTENT OF GRAIN

Mean protein content	Stomatal characters	r
	Frequency	
3 tests	Terminal leaves, means of three tests	−.46*
3 tests	Terminal leaves, means of two tests	−.12
5 tests	Terminal leaves, means of two tests	−.48*
7 tests	Terminal leaves, means of three tests	−.33
5 tests	Seedling leaves, means of two tests	−.32
	Length	
3 tests	Terminal leaves, means of three tests	+.38*
3 tests	Terminal leaves, means of two tests	−.23
5 tests	Terminal leaves, means of two tests	+.29
7 tests	Terminal leaves, means of three tests	+.34
5 tests	Seedling leaves, means of two tests	+.45*

* *Exceeds the 5% point.*

General Discussion

Possibly the most significant phases of the investigation reported herein are first, the correlation studies between stomatal data and those on yield and protein content; and second, the investigation of the possibility of using determinations made on seedling leaves for selection purposes in breeding material.

Results of replicated field trials, conducted over a three-year period, were used as a measure of drought resistance, and stomatal data were obtained from tests that were independent of those from which yield results were procured. Under these circumstances the evidence of a relationship between length of stomata and yield (drought resistance) may be accepted with confidence.

Protein content can be measured quite accurately, as can length of stomata, while the measurement of the yielding ability of a variety is subject to a high degree of error. This being the case and since there is a negative genetical relation between protein content and yield, it may well be that a positive correlation between protein content and stomatal length is a better measure of the relation that exists between yielding ability and stomatal length than is a negative correlation between yield and length of stomata.

The reliability of the correlation coefficient as a measure of a relationship is limited. Among other things, a tendency of varieties to "group" in a scatter diagram may reduce the value of the coefficient obtained. This danger was particularly great in the present study as a number of strains from each of two crosses were included among the varieties on which determinations were made. This was realized and, in cases where a spurious correlation was indicated, a coefficient was not calculated.

Higher correlations were obtained between stomatal length and both yield and protein content when seedling leaf determinations were used than when

the determinations used were made on terminal leaves. This suggests that length of stomata on seedling leaves may prove of value in selecting high yielding strains in breeding material. The plants on which seedling determinations were made were grown in flower pots, and it is difficult to maintain a group of these under uniform conditions in the greenhouse. Improved methods aiming at a more uniform environment are being considered. Such improvements should result in more consistent results between different sets of determinations.

In order to determine more exactly the degree of relationship between yield and stomatal length it will be necessary to use a group of unselected strains from two or more crosses. The data obtained in this investigation make it possible to choose intelligently parental varieties for such a study.

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STUDIES ON THE BIOLOGY OF *ERYSIPHE GRAMINIS* DC.[1]

By W. J. Cherewick[2]

Abstract

A study has been made of various aspects of the biology of *Erysiphe graminis* DC., including its distribution and prevalence in Canada, method of overwintering, physiologic specialization, and the effects of various environmental factors on the development of different stages of the organism. The fungus causes the powdery mildew of cereals and certain grasses. It occurs in every province of Canada, but is more prevalent in British Columbia and the five eastern provinces. The evidence indicates that the fungus overwinters as mycelial mats on dead straw and as mycelial infections on overwintering hosts. Three new physiologic races of barley mildew have been identified, making a total of seven races isolated in Canada. In wheat mildew only Race 1 was isolated. A study of the stability of six races of barley mildew showed that they are distinct biologic entities comparable to physiologic races in cereal rusts. No evidence was obtained by cross inoculations that any variety of *E. graminis*, except possibly *E. graminis Agropyri*, can attack a non-suscept, or an immune variety of its host, or that injury of such hosts renders them susceptible. Conidia of *E. graminis* germinate on immune hosts and penetration proceeds as far as the papilla stage. Physiologic resistance manifests itself in some hosts by the death of the infected cells and in others by the distortion of the haustoria. The evidence indicates that the organism is homothallic. The cardinal temperatures for the germination of conidia are 0°, 10°, and 35° C., while the best development of mildew occurs between 15° and 20° C. Alternating temperatures induce perithecial development. Light is not an important factor, except indirectly, in the development of *E. graminis*. Alternate drying and wetting of perithecia is necessary to induce the formation of ascospores, but other stages are favoured by relatively dry conditions. Conidia germinate well even at zero humidity. Sprinkling infected seedlings with water checked the development of the disease. The addition to the soil of certain fertilizers and other chemicals commonly claimed to increase host resistance to powdery mildew did not increase the resistance of susceptible seedlings of wheat and barley.

Introduction

The extensive literature on powdery mildew of cereals and various grasses, caused by the fungus *Erysiphe graminis* DC., leaves no room for doubt that this organism is of considerable economic importance in many parts of the world. Dimitriou (10) stated that, between 1885 and 1889, the cultivation of cereals in the neighbourhood of Stockholm was seriously threatened by the prevalence of mildew. According to Vik (67), the yield reduction due to mildew sometimes amounts to 30 or 40%. He mentioned (67) that, in Norway, powdery mildew is believed to have caused a heavier reduction in yield than any other fungus disease of the crop. Both in Denmark and Germany (45), powdery mildew has been responsible in recent years for severe losses in spring barley crops, because of the increased area sown to fall barley on which the fungus overwinters. Henrick (18) reported an outbreak of mildew on wheat in great severity in Tasmania in 1934. Hopkins

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(23) pointed out that, in Southern Rhodesia, mildew seriously affected the yield of barley. In 1920, powdery mildew caused more loss than rust in Ohio (62). Many other reports (21, 23, 32, 36) emphasize the importance of the disease caused by *E. graminis*.

In Canada, powdery mildew has received little attention up to the present time, although the disease is apparently as important as in other countries (10). Even in the Prairie Provinces, where the disease is not so prevalent as in other parts, infection ranging up to 85% has been observed.

A considerable number of discrepancies occur in the literature on powdery mildew of cereals, particularly in connection with the biology of the organism. Some investigators (9, 10, 13) concluded that the perithecia serve to carry the fungus over winter, whereas others (15, 36, 45) found the fungus overwintering in the conidial or mycelial stage. Some (12, 21) found that *E. graminis* develops best during a dry season and others (26) claim that the disease occurs only in wet seasons. According to some investigators (2, 57), the weakened state of a host plant favours or is a necessary predisposing factor in mildew infection, while others (63, 64) come to the opposite conclusion. Other contradictory evidence occurs (44, 51; 11, 73; 16, 70), all of which clearly indicates that, in certain aspects, the biology of *E. graminis* either differs under different environmental conditions or is not well understood.

As the importance of *E. graminis* on this continent comes to be more fully appreciated, the work of controlling this organism and of breeding crops resistant to its attacks is likely to be extended. All such work should be based on accurate knowledge of the biology of the organism itself. It was, therefore, with the purpose of exploring further the biology of *E. graminis* that the present investigation was undertaken.

Distribution and Prevalence of *E. graminis* in Canada

E. graminis occurs in different countries and under different climatic conditions in these countries (18, 20, 36, 57, 66, 67), its distribution being apparently almost co-extensive with that of its hosts. In Canada it has been reported in every province from Nova Scotia to British Columbia*, and as far north as Arctic Red River (41), which is approximately 65 miles north of the Arctic Circle. Although no systematic survey of the hosts of *E. graminis* in Canada has yet been made, the organism has been found not only on all the common cereals, but also on *Agropyron* spp., *Beckmannia syzigachne* (Steud.) Fern., *Bromus* spp., *Dactylis glomerata* L., *Hordeum jubatum* L., *Phleum pratense* L., and *Poa* spp.

From various reports and observations, it appears that *E. graminis* is most prevalent in British Columbia and the eastern provinces of Canada. In the three Prairie Provinces, some local infection on barley and wheat occurs

* *Annual Reports of the Canadian Plant Disease Survey, 1920-1942, Division of Botany and Plant Pathology, Department of Agriculture, Canada.*

every year, but only occasionally have general epidemics been experienced. Since, in these provinces, susceptible hosts as well as the organism are present every year, sporadic outbreaks of powdery mildew on cereals apparently depend largely on environmental factors.

Method of Overwintering

An outbreak of powdery mildew is dependent on the presence of inoculum, and the question arises as to whether the inoculum is of local or distant origin. As the conidia of *E. graminis* are rather short-lived (8, 30), long distance dissemination of them would not seem possible. Pape and Rademacher (45) found that wind-borne infection may occur up to several hundred metres, depending on the size of the field serving as the source of inoculum. Observations by the author indicate that the ascospores are even shorter lived than the conidia. The presence of a local supply of inoculum in the spring, therefore, appears to be one of the determining factors in the establishment of an epiphytotic of powdery mildew in any part of Canada, and hence the method by which the organism overwinters requires consideration.

Dimitriou (10) concluded that, in Quebec, *E. graminis* overwinters in the perithecial stage, a finding in agreement with that of Foster and Henry (13) in Alberta. Dash (9) stated that the "ascospores require to pass a winter before they will germinate." On the other hand, Mehta (36) pointed out that the prevalence of powdery mildew on cereals in the Kumaon Himalaya and in the neighbourhood of Simla appears to be mainly due to the survival in the Hills of the conidial stage of the fungus. Several investigators (15, 21, 45) have shown quite conclusively that, in Europe, *E. graminis* overwinters in the mycelial stage on volunteer or winter grain plants.

Careful observations and experiments were made during the last two years to determine in what stage *E. graminis* overwinters in Manitoba. In 1941, a general epidemic of powdery mildew occurred on wheat and barley in this province. Perithecia began to develop early in June and were shedding ascospores early in September. By October 21, all perithecia found on stubble or scattered bits of straw in the field were empty. The late summer and fall was very wet and mild, conditions that evidently favoured the development and expulsion of ascospores. In 1942, a cool wet period occurred in August, followed by comparatively dry and cool weather in September and October, until general freeze-up. By the middle of October, approximately 50% of the perithecia of *E. graminis* produced and expelled their ascospores. Perithecia in which ascospores did not form in the fall remained viable until late in the spring of 1943. Overwintered perithecia were examined on June 14 and some of them still contained asci with undifferentiated protoplasm. Wheat plots growing adjacent to a plot of wheat stubble bearing an abundance of viable perithecia remained, however, completely free from infection throughout the summer.

To determine whether or not the mycelium of the fungus can survive the winter, plots of winter wheat and barley were inoculated with *E. graminis*

late in the fall of 1941. In addition, a plot of naturally infected *Agropyron repens* on which no perithecia could be found before general freeze-up was available for observation. Infected leaves of the wheat and barley bearing mycelial mats were brought into the greenhouse in November, and conidia produced by these infected healthy seedlings of the respective hosts, thus proving that the mycelium was still viable. In the spring of 1942, powdery mildew appeared in the plots of wheat, barley, and *A. repens* early in May, as soon as new growth developed.

In another experiment, barley leaves with thick mycelial mats of *E. graminis Hordei* were collected in the greenhouse before any perithecia developed. They were kept outside from February 9 to March 16, then taken inside the laboratory for five weeks, and finally put in a refrigerator until July 22. The mycelium remained viable all that time, as conidia produced by it infected barley seedlings. Similarly infected barley leaves were alternately frozen at from −15° to −18° C. and thawed at about 25° C. during four consecutive days, being kept outside overnight and in the laboratory during the day. The viability of the mycelium decreased progressively, and by the fifth day only a few conidia were produced when the leaves were placed in a moist chamber. This experiment was repeated, but instead of transferring the material directly from laboratory to outside temperatures, and vice versa, it was placed in a refrigerator at 6° C. for a few minutes before each transfer. The mycelium was still viable when tested after 21 days of freezing and thawing. Furthermore, leaves with mycelial mats in which perithecia were present, were stored in a refrigerator at approximately 6° C. Two years later the mycelium was still viable, as shown by the fact that, when it was placed in a moist chamber, it produced conidia. Similar mycelial mats on dead wheat straw that remained in the field all winter have also been observed to produce conidia.

Foëx (12) concluded that the thickenings in the cell walls of hyphae that he observed in old mycelia on dead leaves of plants serve as the overwintering stage of mildew. This was confirmed in the case of *Microsphaera quercina* (Schw.) Burr. on oak by Petri (46), who found on dead oak leaves hyaline sphaeroidal bodies with a thick smooth or slightly wrinked membrane. These bodies he called "chlamydospores."

To ascertain if thickenings or so-called chlamydospores are present in *E. graminis*, mycelial mats on dead leaves of wheat and barley were stained in aceto-carmine and examined under a microscope. The presence of any definite thickenings could not be confirmed, but it was noticed that certain cells or parts of hyphae and the conidiophores with immature conidia became stained while other parts of the hyphae remained unstained. After alternately freezing and thawing several times, only a few such cells and most of the young conidiophores became stained. It may be that the conidiophores and certain cells are resistant to pronounced fluctuations in temperature and that they are the parts of the fungus referred to by Foëx.

During the fall of 1942, it was noticed that perithecia were expelling ascospores on a mild day in November after about six weeks of freezing weather. It was also noticed that, on green leaves of *A. repens*, the mycelial infections were producing conidia as soon as the temperature went above the freezing point. This observation is in agreement with that of Montemartini (37), who found that, in Italy, *E. graminis* can produce conidia even after a heavy snowstorm. Moreover, it was found in Germany that even new infections are produced during the winter (45).

Further observations are necessary before it can be conclusively determined how *E. graminis* overwinters, but the experimental evidence indicates that, under ordinary conditions, at least in the vicinity of Winnipeg, it may occasionally overwinter to some extent in the perithecial stage, but that it usually overwinters as mycelial mats on dead straw or as mycelial infections on volunteer or winter grain plants and on perennial grasses.

Studies on Physiologic Specialization

Since 1902, when Neger (39) recognized physiologically specialized forms in the conidial stage of certain of the *Erysipheae*, physiologic specialization in *E. graminis* has been studied in different countries and has been definitely established. Marchal (34, 35), using both conidia and ascospores, established seven "physiologic species" in *E. graminis*. His work was soon confirmed by Salmon (50) and Reed (48). Salmon found that one of the so-called species, *E. graminis Bromi* March., could be further subdivided into five "biologic forms", and Reed concluded that there were more than one "at least partially differentiated physiologic forms of mildew" on species of *Poa*. There is no doubt that both Salmon and Reed were dealing with what are now called physiologic races of the respective varieties of *E. graminis*. Mains and Dietz (33) were the first to show the presence of physiologic races in *E. graminis Hordei*. In 1930 they described five physiologic races on barley in the United States and, in 1937, Tidd (63) described two additional races. In Germany, Honecker (21, 22) described nine races of *E. graminis Hordei*, and Schlichtling (56) six races of *E. graminis Tritici*. In Canada the original study of physiologic specialization in *E. graminis* on barley and wheat was done by Newton*, who isolated and identified Race 1 of *E. graminis Tritici* and Races 1, 3, 4, and 6 of *E. graminis Hordei*.

Physiologic Races in Canada

E. graminis Hordei

Recent studies of several mildew collections, as well as of a number of single ascospore cultures of *E. graminis Hordei*, revealed that the original four varieties of barley used by Mains and Dietz (33) as differential hosts, together with the Heil's Hanna C. I. 682, which was added by Tidd (63), are not adequate to differentiate the races prevalent in Canada—just as they were

* *Unpublished data by Dr. Margaret Newton, Dominion Laboratory of Plant Pathology, Winnipeg, Man.*

found inadequate by Honecker (22) for differentiating races prevalent in Germany. In the present investigation, three new races (Races 8, 9, and 10) were isolated in 1941; two of these (Races 8 and 9) could not be differentiated from the other races without including Chevron C.I. 1111 as a differential variety. The infection types of the new races are given in Table I.

TABLE I

MEAN INFECTION TYPES* PRODUCED BY THE THREE NEW PHYSIOLOGIC RACES OF *E. graminis Hordei* ON SEEDLING LEAVES OF BARLEY DIFFERENTIAL HOSTS

Host	C.I. No.	Physiologic race		
		8	9	10
Black Hulless	666	3	1–2	1–2
Chevron	1111	3–4	4	0–1
Goldfoil	928	0	0	0
Heil's Hanna	682	3–4	4	4
Nepal	595	3–4	1–2	1–2
Peruvian	935	0–1	0–1	3+

*0 = *immune or highly resistant, no visible mycelium.*
1 = *very resistant, only slight development of mycelium.*
2 = *moderately resistant, moderate development of mycelium, and slight sporulation.*
3 = *moderately susceptible, moderate development of mycelium, and moderate sporulation.*
4 = *very susceptible, abundant development of mycelium and sporulation.*

E. graminis Tritici

A number of collections of wheat mildew from British Columbia, Manitoba, and Ontario were cultured; all of these yielded Race 1 only.

E. graminis Avenae

Powdery mildew of oats is known to occur in Eastern Canada and in British Columbia, but has never been collected in the field in the Prairie Provinces. Furthermore, as difficulty has been encountered in obtaining viable mildew cultures from distant points, physiologic specialization in *E. graminis Avenae* has not been given attention.

STABILITY OF RACES

Physiologic specialization in *E. graminis*, particularly in the variety *Hordei*, has been studied by several investigators (21, 22, 33, 56, 63). A number of races have been determined, but, as far as the writer is aware, no one has investigated the stability of physiologic races in this species. With respect to cereal rusts, Stakman (61) stated that "physiologic races often are as stable as species themselves." In general, this view is still valid notwithstanding the occasional occurrence of mutations in rust fungi (42). The occurrence of a sudden genetical change in a clonal culture of a race does not alter the fact that the race itself remains a distinct biologic entity. But the expression of pathogenicity in physiologic races of cereal rusts may be influenced by

environmental conditions (25). It might be expected, therefore, that the expression of pathogenicity in races of *E. graminis* may also be influenced by environmental conditions, since this fungus is an obligate plant parasite as are the rust fungi. Some attention was, therefore, given to the effect of environment on the stability of races of this mildew.

In this study the following experiments were made. First, a pure race (Race 6) of *E. graminis Hordei*, obtained from a single conidiospore culture, was kept in culture in the greenhouse for over two years, and two other pure races (Races 4 and 8) were kept in culture for over one year. Collections were made of mycelial infections of Race 6 and stored from two to six weeks in (*a*) refrigerator at approximately 6° C., (*b*) on the office desk, and (*c*) intermittently for two or three days on the office desk and in the refrigerator. After each of these storage treatments, the collections were cultured again. Single spore subcultures of all the three races were made several times. Neither the original cultures, the subcultures, nor the cultures after various storage treatments showed any change in their infection types on the differential hosts.

Second, six physiologic races of *E. graminis Hordei* on the differential hosts were subjected to different environmental conditions. Seedlings of the differential hosts were inoculated simultaneously with each of the six races of mildew and immediately placed in two adjacent compartments of a temperature-controlled greenhouse, one having a temperature approximately of 15° C. and the other of 25° C. Since the incubation period at higher temperature was shorter by from two to four days, the readings were taken according to the development of the infections—on the 9th to 10th day after inoculation in the warmer compartment and on the 12th to 14th day in the colder compartment. The experiment was repeated three times. The average infection types are presented in Table II. It will be seen from this table that the infection types of each race were modified somewhat by the higher temperature.

TABLE II

MEAN INFECTION TYPES OF SIX PHYSIOLOGIC RACES OF *E. graminis Hordei* ON SEEDLING LEAVES OF BARLEY DIFFERENTIALS AT TWO DIFFERENT TEMPERATURES

Host	Physiologic races											
	3		4		6		8		9		10	
	Temperature in ° C.											
	15	25	15	25	15	25	15	25	15	25	15	25
Black Hulless	3+	3−	4	3	1+	1	4−	3	1	2−	2	3−
Chevron	0−1−	0−1−	0	0	0−1	0−1	4	3−	4−	3−	1−	1
Goldfoil	0	0	0	0	0	0	0	0	0	0	0	0
Heil's Hanna	4	3+	4+	4−	4−	4	4+	4−	4	3+	4+	4−
Nepal	4	3	4	3+	1+	2−	4−	3−	2−	2+	2	3
Peruvian	3+	2	1+	0−1−	1	0−1	1	1	1	1+	3	3−

However, in none of the races were the infection types sufficiently modified to make uncertain its differentiation.

To determine the effect of the complex interaction of temperature, light, and photoperiodism, the same six races were cultured on the differentials at certain intervals through the fall, winter, and spring of 1941-42. The infections were generally weak during December and January, when the greenhouse was covered with ice, thus reducing what daylight there is at that time of the year. In general, however, as far as the differential hosts are concerned, the infection types of the six races were affected approximately to the same degree. The variation in infection types brought about by environment was insufficient to cause undue difficulty in the identification of any of the six races.

From these experiments, it may be concluded that physiologic races of *E. graminis Hordei* are distinct biologic entities, comparable to physiologic races of the cereal rusts.

Cross Inoculations

The phenomenon of "bridging", first proposed by Ward (69) in his study on the brown rust of *Bromus* spp., involves the participation of a host through which a pathogen is enabled to pass indirectly from one host to another between which direct transfer is not possible. This phenomenon has been definitely disproved in the cereal rusts (61) but is still in dispute as far as powdery mildew is concerned. Salmon (52) obtained evidence that certain species of *Bromus* might act as a bridge host between two other species for *E. graminis Bromi*. He also stated (52) that "subinfections" on some species of *Hordeum* may serve as a means of bridging. Homma (20) found that *Triticum dicoccum* var. *farrum* was acting as a bridging species for mildew between *T. vulgare* and *T. monococcum* var. *Hornemanni*. Neger (40) concluded that specialization in *Erysiphaceae* is not sharply delimited and that the attacks of mildew are "more frequent and severe towards the end of the vegetative period when the defence of plants is weakened with maturity." He found that *E. Cichoracearum* from *Sonchus asper* produced only subinfections on *S. oleraceus* in summer, while, in October, it produced vigorous conidial development on that host. The resulting conidia fully infected both species of *Sonchus*.

On the other hand, Newton* obtained only negative results in cross inoculations between wheat, barley, and *Hordeum jubatum* and their respective varieties of *E. graminis*. During the last two years of the present investigation, hundreds of cross inoculations were made, and, with one possible exception, no variety of *E. graminis* was able to attack the hosts of other varieties. The only exception to the negative results obtained in all the cross inoculation experiments, was with *E. graminis Agropyri* from *Agropyron repens*, which gave type (2) to (3) infection on Little Club wheat, type (1)

* *See footnote on Page 56.*

to (2) on Glabron barley, and type (0) on rye. The host range of this variety of *E. graminis* requires further investigation.*

Some investigators have reported that, as a result of injuring the plant tissue, successful cross inoculations are made possible. Salmon (54) was able to culture *E. graminis* on different immune hosts by wounding or otherwise injuring their leaves. He obtained infection even on rhizomes of *Elymus arenarius* with conidia of *E. graminis Tritici*. This work of Salmon was later confirmed by Hammarlund (17). Mackie (32), in his study of barley mildew, found that inoculations on the exposed mesophyll consistently broke down the resistance of the host.

On the contrary, Levine and Stakman, according to Stakman (61), inoculated some 2000 plants of barley, rye, and oats applying "every conceivable form of torture," but could not get any infection by *E. graminis Tritici*. Similar results were obtained by the author from hundreds of cross inoculations on injured host tissue. Leaves of wheat, barley, and oats, wounded by various methods, were inoculated with conidia of all the available races of barley and wheat mildew. In one series of cross inoculations, a susceptible and an immune variety of barley and of wheat were used. The leaves were wounded by (*a*) pricking with a needle; (*b*) burning with a hot needle; (*c*) stripping the epidermis with a razor blade; (*d*) cutting out portions of the epidermis and mesophyll on either side of a leaf, leaving only the opposite epidermis intact. Conidia were placed both on the wounded surface and the opposite side of the leaf. Thereafter, the inoculated seedlings were usually covered with a glass lamp-chimney, on top of which a Petri dish was placed to prevent too rapid drying of the wounds. Each individual treatment was applied to 10 or more leaves. The whole experiment was repeated several times. In no case did a variety of *E. graminis* produce infection on the host of another variety or on an immune variety of its own host.

In another series of experiments leaves were cut off the plant, wounded, and inoculated as before, and placed on a moist filter paper in a Petri dish. Several susceptible and resistant varieties of barley and wheat, and one variety of oats were used. Also, both young and old leaves were taken off seedlings as well as adult plants. Again the results were consistently negative.

Salmon (54) found that susceptibility can be induced not only by mechanical injury, but also by such interference with the normal functions of the plant cells as follows the application of anaesthetics. This was partially confirmed by Stakman (60) when, in his cross inoculations with cereal rusts, he obtained a slight increase in susceptibility in several instances after exposure of cereal seedlings to the vapours of ether or chloroform. In the present investigation, leaves were cut off the plant, exposed to ether or chloroform vapour for from 0.5 to 5 min., or dipped in 10% ethyl alcohol for from 1 to 12 hr., then inoculated, and placed on moist filter paper in a Petri dish. The tests were

** After this thesis was submitted to the Graduate School of the University of Minnesota, J. R. Hardison published an abstract (Phytopathology, 33 : 5, 1943) in which he states that mildew from Agropyron repens produced infection on barley and some other hosts.*

repeated several times, but no infection was obtained. Longer exposure to ether or chloroform vapour evidently killed the cells, as such leaves turned chlorotic within a short time after the treatment.

Finally a series of cross inoculations was made using host tissue extract in various ways. Klika (28) mentioned that host penetration by mildew is due to chemotaxis. Newton, Lehmann, and Clarke (43) reported that infection of susceptible varieties of wheat by rust was in some cases reduced by administering extracts of resistant varieties either in a Petri dish culture or by direct injections. To explore what possibilities lay in this direction, the effect of extracts of susceptible hosts on infection by *E. graminis* of naturally immune grain plants was investigated. The extracts were obtained by grinding the leaves in a small meat-chopper. Several methods of introducing the extract into the leaf tissues were used. Portions of the epidermis of the leaves to be inoculated were removed, the wounds flooded with the extract, and then inoculated. The extract was injected under the epidermis with a fine hypodermic needle and conidia sown over the injected areas. Small seedlings were cut off just above the soil level, placed in a small beaker with a particular extract and subjected to a vacuum of approximately 20 in. of mercury for from one to three hours, after which the leaves were inoculated. The results were again all negative, showing that, under the conditions of these experiments, the races of *E. graminis* that were tested could not be induced to parasitize a naturally immune host.

Studies on Host Resistance

Neger (40) found that resistance to the Erysiphaceae is due to an enclosure of the developing haustoria in the host cells by a gummy substance, thus preventing their further development. Both Corner (8) and Homma (20) demonstrated that the development of powdery mildew on a resistant host is arrested just at the time that the peg from an appressorium penetrates the cell wall, or, according to Corner (8), at the papilla stage. Corner (8) also showed that penetration by *E. graminis* takes place up to the papilla stage on such unrelated plants as *Impatiens Sultani*, *Polypodium* spp., etc., while Neger (40) observed that, on some resistant plants, conidia germinated but no penetration occurred. Graf-Marin (16) found that resistance in older leaves of barley is due to the thickening of the cuticle.

In the present investigation, host resistance was studied in several varieties of both wheat and barley. In an attempt to overcome the long procedure required in the preparation of the standard microtome sections and the difficulty of stripping the epidermis, as used by others (8, 16), it was found that, when infected leaves were bleached in alcohol and stained in aceto-carmine, cell penetration and the development of haustoria could be seen quite clearly. This is illustrated in Figs. 1 and 2. The inoculated leaves were bleached in 70% ethyl alcohol, then placed on glass slides, flooded with aceto-carmine, and left in a moist chamber overnight, or else the slides were heated over a flame almost to the boiling point of the stain, when immediate observation

could be made. As a final check on penetration and infection, both free-hand and microtome sections were studied. Microtome sections, prepared according to the usual paraffin method, were cut 8 and 10 μ thick and stained with Flemming's triple stain (safranin, gentian violet, and orange G).

As a result of the study of many specimens during the cross inoculation experiments, it was found that germination of the conidia, and penetration up to the papilla stage, take place as well on the naturally immune hosts tested as on the susceptible ones. With aceto-carmine, the penetration points stain deeper red than the surrounding tissue, indicating some reaction between the host cell and the penetration peg of the pathogen. A study of the hosts inoculated with races of their own varieties of *E. graminis* revealed two distinct types of resistance. In susceptible varieties of wheat and barley, the haustoria are numerous, large, and with several long, finger-like projections at each end. In some resistant varieties, the haustoria decrease in size and become more abnormal with an increase in resistance until, in type (1) infections, there are only small sphaeroid lobes, which, apparently due to some antagonistic relationship with the cell protoplasm, cannot develop into proper haustoria. Since no change in the cytoplasmic reaction to the stain could be observed, it seems that the inability of the fungus to establish food relationship with the host cell must be due to the physiological composition or reaction of the cytoplasm. The progressively greater resistance in such varieties appears to differ only in degree rather than being a different physiological phenomenon.

In other resistant varieties in which infection points are marked by necrotic spots, the haustoria develop almost as well as in the susceptible varieties. This fact is illustrated in Fig. 2, where a haustorium is shown in a type $(1)^+$ infection. The infected cells soon break down and therefore the fungus is unable to develop any further. The rapidity of the cytoplasmic disintegration after penetration determines the type of infection. In type (;) infections, the cells disintegrate soon after penetration, while, in type (3), the cells are killed only after an advanced development of the fungus. Salmon (52) observed that the haustoria in resistant varieties disintegrate within from one to six days after they develop. It seems, therefore, that the more compatible the host is with the pathogen, the slower is the disintegration of the host cells and the haustoria.

Hurd (24) found that severe infection by *E. graminis* results in an abnormally high acid content of wheat plants. Allen and Goddard (1) showed that, in infected wheat, there is an increase in respiration and fermentation of the mesophyll tissues roughly proportional to the degree of infection. They suggest that a diffusible substance is produced by the fungus or the infected cells, which passes into the mesophyll and causes the rise in respiration. At any rate, there is evidence that something, whether an acid or some other toxic substance, diffuses out of the infected cells, as the author observed that, in plants showing resistance marked by necrosis, not only were the infected epidermal cells gradually killed but also the adjacent mesophyll cells.

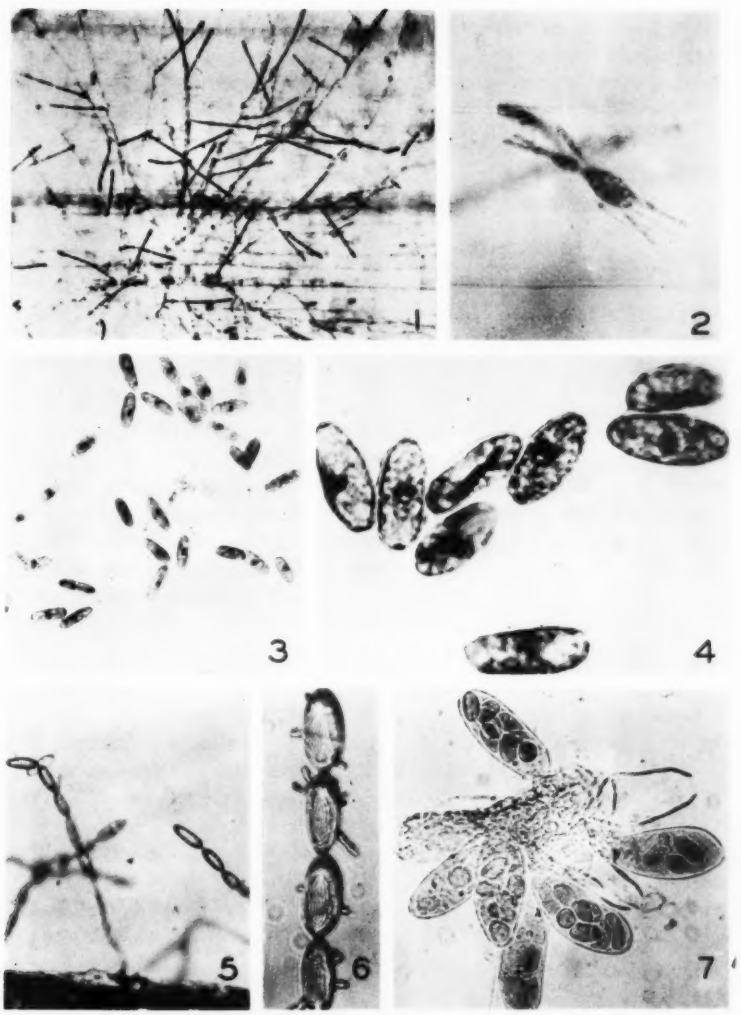

FIG. 1. *A type (1)⁺ infection, stained in toto, showing heavily stained penetration points, and the few conidiophores that usually develop in this type of infection.* × *200.*

FIG 2. *A haustorium developed in the infection shown in Fig. 1, and photographed before the infected cell began to disintegrate.* × *800.*

FIG. 3. *Conidia of Erysiphe graminis stained in aceto-carmine to which a crystal of haematoxylin was added.* × *400.*

FIG. 4. *Conidia of Erysiphe graminis, showing a binucleate conidium (second from left).* × *1200.*

FIG. 5. *Conidia of Erysiphe graminis, one germinating in situ.* × *400.*

FIG. 6. *Part of a conidial chain removed from a conidiophore to facilitate higher magnification of germinated spores.* × *800.*

FIG. 7. *A group of asci in different stages of ascospore development, from a perithecium of E. graminis stored for two years.* × *400.*

Studies on Sexuality of *E. graminis*

Yarwood (70) showed that *E. Cichoracearum* DC. on sunflower is heterothallic. Homma (19) found that *Sphaerotheca fuliginea* (Schlecht.) Pollacci on *Taraxacum ceratophorum* is homothallic. Both of these authors based their results on single conidiospore cultures. Newton* studied many single conidiospore cultures of *E. graminis Hordei*, but, as the factors that stimulate the development of perithecia were not controlled, her results were uncertain, although she had some indications that the fungus is heterothallic.

In the present investigation, the study on the sexuality in *E. graminis* was prompted by two definite motives. At the very outset it was realized that a method of storing mildew cultures would be of great advantage because carrying different races of the organism in culture in a greenhouse throughout the summer is very difficult. It was found that mildew perithecia could be stored for some time, and, therefore, it was necessary to determine if perithecia would develop in single-spore cultures, thus making available a particular race in pure form. The second motive was to determine whether *E. graminis* is homothallic or heterothallic, with the hope of throwing some light on the problem of physiologic specialization.

The first essential for this study was, of course, to start a culture from a uninucleate spore. Since the nuclear condition of the conidia was not known, a large number of them were subjected to examination. The conidia were dusted on to a slide lightly smeared with egg albumen. The slide was then flooded with aceto-carmine; a small crystal of haematoxylin was added; and the slide was placed in a moist chamber to prevent evaporation of the stain. At ordinary room temperature, nuclei became well stained in from 18 to 24 hr., but slight warming of the slide gave as good results in from four to six hours. This staining method is very simple and gives good nuclear differentiation, as is shown in Figs. 3 and 4.

The conidia of all the varieties of *E. graminis* studied were mostly uninucleate, but there usually was about 2% of binucleate spores. The nuclei in the binucleate spores, however, were always smaller in size, and very close together. The smaller size and close proximity of the two nuclei indicated that they had originated as a result of an ordinary mitotic division. Occasionally an abnormally long conidiospore was found that contained two widely separated nuclei. Such a spore was evidently a double spore, resulting from the failure of constriction. These double spores are not common, and, because of their size, were easily avoided in making single-spore isolations.

Ascospores of wheat and barley mildew were also examined and found to be uninucleate. Therefore, in this study, both single-conidiospore and single-ascospore cultures were regarded as having arisen from uninucleate spores.

Since the fall of 1941, 29 single-spore cultures of *E. graminis Hordei* and 4 of *E. graminis Tritici* have been studied and of these 27 have produced perithecia. The single-spore cultures were kept either in separate compart-

* *See footnote on Page 56.*

ments of a greenhouse or in isolation booths. These booths were enclosed with glass on three sides and glass or cellophane on top, the fronts being covered with white cotton cloth. Three single-spore cultures of barley mildew belonging to Race 6 and three of the wheat mildew failed to produce perithecia. Race 6 of barley mildew and Race 1 of wheat mildew, however, rarely produce perithecia in the greenhouse. In all other single-spore cultures, perithecia developed within from four to six weeks and, in a few cultures, perithecia appeared on several of the originally inoculated seedlings. On several occasions, perithecia of the single-spore cultures were collected and from these ascospore cultures were subsequently established. In each case a pure race of the respective parent culture was obtained.

These results indicate strongly that at least certain varieties and races of *E. graminis* are homothallic. On the other hand, conidial collections of barley mildew made during the last two years from the experimental field plots yielded only Races 4 and 6, while collections of perithecia from the same plots yielded Races 4, 6, 9, and 10. These results seem to indicate that recombination and segregation of factors occur in perithecia developed in the field. If this is true, it makes the problem of sexuality in *E. graminis* more complicated. Further studies are necessary before definite conclusions may be reached regarding this point.

Studies on the Effect of Environment Upon the Pathogen and the Disease

A study was made of the effect of environmental conditions upon the pathogen and the disease. In addition to general observations made in the greenhouse and in the field on the effect of environment upon powdery mildew, a number of experiments were made on seedling plants in the greenhouse. The seedlings were grown in 4-in. pots, usually with from 20 to 30 seedlings per pot. The inoculations were made by shaking heavily infected plants over the seedlings, which were then placed under the required conditions.

Effect of Temperature

Temperature and Storage of Conidia

Little is said in the literature relative to the longevity of the conidia of *E. graminis*. Corner (8) noted that the conidia remained viable from two to three days, and even as long as one week. Levykh (30) found that conidia of tobacco mildew (*Oidium tabaci* Thuem.) survived for 12 days at a temperature of 14° C. and an air humidity of from 80 to 89%.

Experiments on the longevity of the conidia of *E. graminis* are rather difficult, because, as will be shown presently, not only do the spores germinate in dry air and at temperatures as low as freezing point, but also *in situ*—that is to say, while still attached to the conidiophores. Observations by Brodie and Neufeld (3) indicated that the conidia of *E. Polygoni* DC. do not germinate *in situ*. Under some conditions, however, they do. The author kept infected

plant tissue bearing mature conidia of this fungus at approximately 3° C. and 100% relative humidity and observed germination of the conidia *in situ*. The conidia of *E. graminis* have been observed to germinate *in situ* both in the greenhouse and out-of-doors (Figs. 5 and 6). In the greenhouse at a temperature of from 10° to 15° C., up to 32% of the conidia have been found germinated, a fact necessitating special precautions in the study of their longevity. For example, spores used for storage tests could only be collected when no freshly germinated ones occurred and those that had germinated a day before were already shrivelled.

In one test about 1 cc. of conidia of *E. graminis* were collected in a cellophane capsule and immediately put into a refrigerator at approximately −4° C. A sample was taken out at weekly intervals for a germination test during a period of six weeks. On the 42nd day of storage, very few of the spores remained viable, probably because each time the sample was taken the spores in the capsule thawed and, on account of the repeated freezing and thawing, were killed. The test was repeated by dusting conidia on to dry slides and storing them at approximately −7° C. One slide was taken out at weekly intervals for a germination test. The test was interrupted after three weeks of storage, because, during the defrosting of the refrigerator, some of the conidia germinated, thus making further germination tests unreliable. The percentage of germination in these two tests is shown in Table III.

TABLE III

THE LONGEVITY OF CONIDIA OF *E. graminis* STORED AT −4° C. TO −7° C. AND TESTED AT WEEKLY INTERVALS

Length of storage, wk.	Percentage of germination	
	In capsule at −4° C.	On dry slides at −7° C.
Initial	63	47
1	37	33
2	31	34
3	29	25
4	11	—
5	—	—
6	Trace	—

In still another test, dry slides dusted with conidia were stored at approximately −7° C. for nine days, during which time they were taken out at two-day intervals into room temperature for a few minutes. On the ninth day, only 11% of the barley and 3% of the wheat mildew conidia were still viable. On one occasion dry slides with conidia were left outside overnight when the temperature went down to −25° C., with no appreciable injury to their viability. These tests indicate that conidia of *E. graminis* are not readily killed by freezing, but are rather easily killed by alternating freezing and thawing. At temperatures above freezing, storage of the conidia of *E.*

graminis is impossible, because they either germinate or shrivel up and die regardless of the relative humidity.

Temperature and the Germination of Conidia

Graf-Marin (16) showed that conidia of *E. graminis* germinated at temperatures of from 3° to 20° C., the optimum being 15° C. Corneli (7) found the cardinal temperatures for germination of the conidia to be 5° C., 18° to 20° C., and 27° C.

In the present investigation, a range of temperatures from zero to 35° C. was used for testing the germination of conidia of *E. graminis*. Spores were dusted on to dry glass slides that were immediately placed on moist filter paper in Petri dishes and kept at the temperatures indicated in Table IV.

TABLE IV

THE EFFECT OF TEMPERATURE ON THE GERMINATION OF CONIDIA OF *E. graminis** AT 100% RELATIVE HUMIDITY

Temp., ° C.	Percentage of germination after 24 hr.			
	E. graminis Tritici		*E. graminis Hordei*	
	Range	Mean	Range	Mean
0	6 −39.0	9.0	3−41	8.2
3	5 −79.0	17.0	7−63	21.0
6	7 −79.0	56.0	5−71	46.9
10	11 −97.0	57.5	6−75	42.3
15	10 −81.6	41.8	10−41	28.7
20	5 −83.6	40.5	3−68	22.5
25	7 −67.0	23.3	3−49	13.4
30	0.5−14.0	9.1	2− 8.3	4.7
35	0 − 1.0	Trace	0− 1	Trace

** Germination of conidia of E. graminis Avenae and E. graminis Agropyri was also tested once, and the results were much the same as for the two varieties given in the table.*

Temperature control tanks were used to secure temperatures between 10° and 35° C., and refrigerators for the lower temperatures. In each test, an extra slide dusted with spores was immediately examined under the microscope to determine the percentage of shrivelled spores and those that had germinated *in situ*. This percentage was used as a correction factor in determining the percentage of spores that germinated during the test. For maximum and minimum germination temperatures, the slides were first examined under the microscope before the test, and only fields on the slide with normal ungerminated spores were selected and marked for germination counts. These precautions were found necessary to ensure that shrivelled spores or those germinated *in situ* were not included in the final results. The average percentage of germination, based on a count of from 300 to 500 spores per slide in five separate tests, is given in Table IV.

As will be seen from Table IV, conidia of *E. graminis* germinate well at from 3° to 20° C., but the extreme range is from 0° to 35° C. The optimum is not well defined owing to the fact that the type of germination varied at different temperatures. At lower temperatures, the conidia germinate with one or sometimes two germ tubes, which develop slowly but remain alive for several days, some of them eventually attaining a length 10 times that of the spore. At higher temperatures, the conidia germinate with from three to nine germ tubes, some of which attain similar length in one day, but they perish much sooner. Moreover, the optimum seemed to vary from one test to the other. After a bright day, when the percentage of germination was high (over 80%), the optimum appeared to be between 15° and 20° C., whereas, in dull weather, when the germination was weak, the optimum seemed to be about 10° C. From the mean percentages of germination, it appears that the cardinal temperatures are 0°, 10°, and 35° C. It should be noted that conidia of all the four varieties of *E. graminis* germinated in a refrigerator, where the moist filter paper and the condensed moisture in the Petri dish froze solid.

Temperature and the Development of the Disease

Not many observations have been recorded on the effect of temperature on the development of powdery mildew on cereals. Hammarlund (17) found 15° to 20° C. as optimum for the development of *E. graminis Tritici*, a result confirmed, in general, by Corneli (7) and Honecker (21). The range of temperatures, however, under which mildew can thrive appears to be rather wide. Corner (8) observed that mildew thrived during a hot dry summer in England when other parasitic fungi suffered from drought, while Montemartini (37) found mildew active in winter in Italy, both of which observations are in accord with those of the author in Manitoba. Cultures kept in the greenhouse during the summer months were able to survive temperatures up to 43° C., so long as there were alternating cooler periods, and, as mentioned before, mildew on *Agropyron repens* was observed to produce conidia in November at temperatures just above freezing. The cardinal temperatures could not be determined owing to lack of proper facilities, but the results of a few experiments indicate that both infection and the development of the disease is best at from 15° to 20° C.

In one experiment, 20 pots of Glabron barley seedlings were inoculated with a mixture of mildew races. Four pots were immediately placed in a hot greenhouse at approximately 26.5° C., and the other 16 pots left in a cold house at about 18° C. Of the 16 pots, four were kept in the cold house; four were carried daily to the hot house in the morning and back again to the cold house in the evening; and the remaining eight pots were transferred to the hot house two at a time, one, three, five, and seven days after inoculation. The experiment was repeated three times, with the results summarized in Table V.

It may be seen from Table V that the greatest spread of mildew took place under the alternating temperatures, but the individual pustules soon developed mycelial mats in which perithecia formed. At the continuous low tempera-

TABLE V

THE EFFECT OF TEMPERATURE ON THE INFECTION AND DEVELOPMENT OF MILDEW ON BARLEY SEEDLINGS

Total No. of seedlings tested	Treatment	Incubation period in days*	Mean infection, %	Remarks
260	Continuous at 26.5° C.	7	50	Sporulation weak, little spread through secondary infections
273	Continuous at 18° C.	12	80	Sporulation vigorous, mildew spreading well
258	Alternating	8	85	Spreading very well, life of individual pustule short
214	At 18° C. 1 day, then at 26.5° C.	9	35	Some sporulation and little spread
203	At 18° C. 3 days, then at 26.5° C.	10	40	Some sporulation and little spread
225	At 18° C. 5 days, then at 26.5° C.	10	40	Some sporulation and little spread
197	At 18° C. 7 days, then at 26.5° C.	12	50	Sporulation weak, little spread

* *The incubation period was counted from the time of inoculation to the time of appearance of conidia.*

ture, development was slower but the pustules continued a vigorous production of conidia for from 20 to 30 days, when the pots were discarded. At the continuous high temperature, even on seedlings that were infected in the cold house, mildew sporulated only for a short time, and then turned into thin mycelial mats without producing perithecia, a result in accord with observations of Newton*.

In another experiment, six races of barley mildew were tested at two different temperatures. Four pots each of Glabron barley seedlings were inoculated with Races 3, 4, 6, 8, 9, and 10, two of which were put in the hot house and the other two in the cold house. The experiment was repeated several times, with the average results presented in Table VI. Again, mildew development was much more vigorous in the cold house, although there was some variation between different races. Race 6 seemed to thrive almost as well in the hot house as in the cold. It is a common race in the Prairie Provinces.

Temperature and the Formation of Perithecia

Dash (9) observed that perithecia are formed in the fall, when the vitality of the host plant is low. Graf-Marin (16), in his study of the factors affecting the formation of perithecia, concluded that perithecia are formed only on

* *See footnote on Page 56.*

TABLE VI

THE EFFECT OF TEMPERATURE ON THE INFECTION AND DEVELOPMENT OF SIX RACES OF POWDERY MILDEW ON BARLEY SEEDLINGS

Race	Incubation in days		Mean percentage of infection	
	At 26.6° C.	At 18° C.	At 26.6° C.	At 18° C.
3	6	9	25	55
4	7	7	50	76
6	5	9	50	55
8	7	10	10	50
9	7	7	60	76
10	6	7	60	90

old leaves and old plants, the environmental factors having no effect. Laibach (29) found that the development of perithecia is primarily dependent on the genotype of the host. Light, humidity, and temperature, he concluded, were only indirect factors, acting upon the host and thus creating unfavourable conditions for the assimilation of food by the fungus. Salmon (53), on the other hand, found that, in *Sphaerotheca Humuli* var. *fuliginea* (Schlecht.) Salm. on *Taraxacum officinale*, perithecia were formed 21 days after inoculation, there being perithecia in a small oidial patch on a leaf that was not yet fully developed. Yarwood (70) showed that conditions commonly cited as favouring perithecial formation, e.g., old leaves, poor nutrition of the host, low temperature, and low humidity, had little or no influence on the production of perithecia in sunflower mildew. Gorlenko (15) observed that, in Russia, perithecia of *E. graminis* are formed in summer and ascospores are shed during August. This agrees with the author's observations made in 1941, when perithecia were noticed early in June. Moreover, perithecia have been observed in Manitoba early in the summer on leaves of wheat not quite fully developed. Such observations are in contradiction to the prevalent idea (12, 16, 20, 28, 29) that perithecia form only on leaves more or less exhausted of food materials. A few experiments were, therefore, made to determine the conditions under which perithecia are initiated.

Several different treatments were tried, such as exposing infected seedlings to various intensities of light, exposing to ultraviolet light, fertilizing with various fertilizers, and chilling in an ice-box at about 5° C. overnight on several successive nights. Perithecia developed on the chilled plants within two weeks after the first chilling, while similarly infected seedlings under various other treatments did not produce any perithecia for from five to six weeks, at the end of which time the plants were discarded. It was then thought that chilling at a low temperature was a stimulating factor.

Later, during an experiment on the effect of temperature on the development of mildew, described in a previous section of this paper, it was noticed that mildew, on seedlings grown under alternating temperatures, developed perithecia three weeks after the plants were inoculated. In another test of

the same experiment, perithecia developed 13 days after inoculation in the hot house, when, during the test, the temperature fell to about 10° C. on two occasions.

Subsequent to these observations, many tests with alternating temperatures were made, and it was found that the wider the fluctuations in temperature, the greater is the production of perithecia stimulated. Just as in the case of cereal rusts, physiologic races differ in the readiness with which they produce teliospores, so do races of barley mildew in their ability to produce perithecia. In the greenhouse, for example, Race 8 produces perithecia very readily, while Race 6 produces them very rarely. With temperatures alternating several times between 40° and 5° C., even Race 6 has been induced to develop perithecia within 20 days after inoculation.

Temperature and the Storage of Perithecia

Field collections of wheat and barley mildew perithecia were stored (*a*) on an office desk, (*b*) in a refrigerator at from 3° to 6° C., (*c*) alternating bi-monthly between the refrigerator and room temperature, (*d*) alternating between outside (as low as −30° C.) and room temperatures. After two years of storage under the various conditions, viability was still not appreciably affected in any of the samples (Fig. 7). It seems, therefore, that dry perithecia are not readily killed by ordinary fluctuations in temperature.

Temperature and the Formation of Ascospores

Graf-Marin (16) found that, when perithecia of *E. graminis* submerged in water were chilled at 9° C. for 12 hr. and then kept at 21° C., ascospores were formed in 22 hr. In the present studies, a field collection of *E. graminis* perithecia, which contained only asci with undifferentiated protoplasm, was treated as follows: (*a*) chilled in water at 6° C. overnight, then left on an office desk; (*b*) put in water and left on the office desk without chilling; (*c*) chilled dry, then put in water, and left on the desk. In all three treatments, ascospores developed on the third day after the perithecia were soaked in water. The test was repeated several times, but there seemed to be no apparent stimulation or hastening of ascospore development by chilling the perithecia. On the other hand, when perithecia collected in the greenhouse and kept on the office desk for a week were tested, ascospores developed sooner and were more abundant in the chilled specimens. Chilling dry and then soaking in water was the most effective treatment of perithecia that had developed in the greenhouse.

Effect of Light

Light and the Germination of Conidia

According to Neger (39), conidia, sown in water and exposed to light from one side only, germinate almost exclusively towards the light, thus exhibiting positive phototropism. He also stated that germ tubes developed in light were thicker and stronger than those developed in darkness. In the present investigation, conidia of *E. graminis*, sown in a drop of water and exposed

to light—both daylight and a 60-watt electric lamp—from one side only, did not show any phototropism. Very few submerged spores germinated, while those on the surface of the water or on the dry slide germinated in every direction. The test was repeated several times with the same results.

In another experiment, the effect of light on the germination of conidia was tested at a range of temperatures from 10° to 35° C. Dry glass slides dusted with the spores and placed in moist chambers (Petri dishes), were put in earthenware crocks in the temperature control tanks, one crock in each tank being covered with an earthenware lid and another one with an ordinary pane of glass. The experiment was repeated three times at all six temperatures and five times at 10° and 25° C. The average percentage of germination is shown in Table VII. From this table, it will be seen that, at higher

TABLE VII

THE EFFECT OF LIGHT ON THE GERMINATION OF CONIDIA OF *E. graminis* AT A RANGE OF TEMPERATURES FROM 10° TO 35° C.

Temp., ° C.	Per cent germination				Remarks
	Wheat mildew		Barley mildew		
	Dark	Light	Dark	Light	
10	62.5	76.9	40.7	46.0	Some of the germ tubes produced in darkness appeared slightly longer and more slender than those produced in light
15	51.1	64.1	28.4	39.3	
20	31.4	44.8	20.8	25.6	
25	27.8	38.9	25.3	21.6	
30	10.7	5.1	6.1	1.7	
35	1	Trace	Trace	Trace	

temperatures, spore germination was depressed by light, while at lower temperatures, it was stimulated. Since the temperatures were carefully checked and found to be the same in each pair of the crocks, the consistent difference in the percentage of germination was evidently due to the influence of light.

Yarwood (72) found a definite diurnal cycle in *E. Polygoni* but not in *E. graminis Hordei*. No diurnal cycle was found by the author in barley or wheat mildew. Spores collected at different periods of the same day showed less variation in their viability than spores collected on different days. After a period of dull cloudy weather during the winter months, the viability of the spores was much lower than after a bright day. The viability of conidia produced under various conditions was tested in the following manner. From 8 to 12 hr. before each test, the infected plants were vigorously shaken, to ensure as far as possible that only conidia produced afterwards were used in in the respective test. The spores were collected by holding the infected plants over the glass slides and gently tapping the plants with a pencil. The slides were placed in moist chambers at approximately 6° C. for 16 to 24 hr.,

at the end of which period the percentage of germination was determined by counting 300 spores per slide. These tests were made during December and April. Data for a representative test are shown in Table VIII.

TABLE VIII

VIABILITY OF CONIDIA OF *E. graminis* PRODUCED UNDER DIFFERENT LIGHT CONDITIONS

Conditions under which spores were produced	Percentage of germination			
	December		April	
	Wheat mildew	Barley mildew	Wheat mildew	Barley mildew
During the night	42	39	58	76
During the day	47	38	63	71
During cloudy weather	3	5	61	62
During bright weather	66	64	83	79

It is realized that, since germination tests during cloudy and bright weather were not made on the same day, other factors might have been operative, but, as the tests were repeated several times, it appears that light was the most important factor. In general, these results confirm the conclusion of Volk (68) that spores produced in a favourable environment are more viable than those produced under adverse conditions.

A few tests were subsequently made in which pieces of infected leaves were floated on sugar solution in a Petri dish and kept in darkness. The solution provided a source of carbohydrate (65). Conidia produced under such conditions germinated up to 90%. The fact that conidia produced at night or in Petri dishes in the dark germinate well, shows that the effect of light on the fungus is not direct but rather indirect. Apparently the unfavourable effect of inadequate light on the fungus is exerted through the host in that low light intensity curtails metabolic activity, and thus the conditions of nutrition are rendered unfavourable to the organism.

Light and Host Infection

No specific report on the effect of light on the infection of a host by *E. graminis* has been found, excepting the preliminary note by Sempio (58), who found that inoculated seedlings placed immediately in darkness after inoculation showed normal infection, but, when placed in darkness on the seventh day after the inoculation, the seedlings became flaccid, with rolled necrotic leaves.

To check the effects of light on the infection by *E. graminis*, inoculated seedlings were kept (*a*) in a dark chamber for from one to three days; (*b*) in the diffused light of a glass booth covered with a double layer of cheese cloth, for from one to three days; and (*c*) exposed to sunlight on an ordinary greenhouse bench. When the temperatures in the dark chamber and the shaded glass booth were kept approximately the same as those on the open bench

(15° to 18° C.), there was no difference either in the percentage of infection or in the time of incubation in any of the light treatments, a result showing that host infection by powdery mildew apparently proceeds in the dark as well as in sunlight.

Light and the Development of the Disease

Light has been found essential for the development of mildew by Hammarlund (17). Volk (68) showed that, under slight shade produced by one layer of gauze, the development of mildew was better than in stronger shading or in full daylight. Trelease and Trelease (65) observed in their field experiments that wheat mildew developed well in the deep shade of trees, but that in full sunlight only flecks developed. Sempio (2) reported that cereal plants exposed to continuous light were resistant to mildew, and, according to Moshkov (38), the optimum daily photoperiod for the development of various mildews was 12 hr., while less than nine hours or more than 17 prevented the development of the disease.

The effect of light on the development of powdery mildew has been under observation in Winnipeg for several years. It was noticed that, each winter during the period of short daylight in December and January, mildew did not develop well, particularly when there were a considerable number of cloudy days. These observations were checked by experiments made in April, when, under ordinary conditions in the greenhouse, mildew development seems to be at its best. Seedlings of wheat and barley were inoculated and immediately placed in five glass chambers having different degrees of light, namely, (*a*) an ordinary glass chamber, (*b*) a glass chamber covered with one ply of cheese cloth, (*c*) another one covered with two ply of cheese cloth, (*d*) a fourth covered with brown paper on top and two ply of cheese cloth on the sides, and (*e*) a dark chamber in which the seedlings were kept up to four days and then under two ply of cheese cloth. The greenhouse was kept at approximately 18° C. and the temperature in all the chambers was the same. The experiment was repeated twice, and no apparent difference in the development of the disease could be noticed in any of the chambers. Even complete darkness for four days did not retard the development of *E. graminis*.

In another experiment, wheat and barley seedlings were kept in the five chambers for four days before inoculation. They were then inoculated and left in their respective chamber, except that the seedlings from the dark chamber were removed to the chamber under the brown paper and cheese cloth cover. The results of this experiment are presented in Table IX. It will be seen that seedlings kept in the dark before inoculation became quite resistant to mildew, a fact that confirms the results obtained by Trelease and Trelease (65). The susceptibility of the seedlings in the above experiment did not seem to be adversely affected by shading with cheese cloth alone, probably due to the fact that plants seem to grow normally even in diffused light during bright April days.

TABLE IX

SUSCEPTIBILITY OF WHEAT AND BARLEY SEEDLINGS KEPT FOR FOUR DAYS IN DIFFERENT LIGHT INTENSITIES BEFORE INOCULATION WITH *E. graminis*

No. of seedlings tested		Light intensity	Type of infection		Per cent infection	
Wheat	Barley		Wheat	Barley	Wheat	Barley
71	66	Full daylight	4	4	65	70
76	69	Under 1-ply cheese cloth	4	4	72	70
64	73	Under 2-ply cheese cloth	4	4	63	60
70	60	Under brown paper	3	3	48	35
72	64	Dark chamber 4 days	1–2	2	10	5

The effect of photoperiodism was tested in an experiment during the latter part of November and the first half of December. Inoculated seedlings of wheat and of barley were each divided into four similar groups, which were exposed to the following photoperiods: (*a*) continuous light; (*b*) 13 hr. of light daily; (*c*) nine hours of light daily; (*d*) seven hours of light daily. Another set of seedlings were subjected for four days before inoculation to the same four light periods. The daylight was supplemented by an ordinary 500-watt lamp with white porcelain reflector, held 36 in. above the tops of the seedlings. The results, summarized in Table X, indicate that different photoperiods had no direct effect on the development of the fungus, as the infections developed normally in the plants that were inoculated before they were exposed to the different photoperiods. On the other hand, the plants

TABLE X

THE EFFECT OF DIFFERENT PHOTOPERIODS ON THE SUSCEPTIBILITY OF WHEAT AND BARLEY SEEDLINGS TO *E. graminis*

No. of seedlings tested	Length of daily light period	Infection type	
		Inoculated at the beginning of experiment	Inoculated four days later
127	Continuous	4	0
93	13 hr.	4	4
61	9 hr. (normal day)	4	3
117	7 hr.	4	1

inoculated four days after they were subjected to the different photoperiods, showed different results. Seedlings that received continuous light or only seven hours of light daily became resistant to mildew. Moreover, in all the seedlings exposed to the continuous light and in those that received daily seven hours of light, the leaves that developed during the experiment did not

become infected in spite of daily dustings with conidia. These results show that apparently the very short photoperiods and the continuous light caused some physiological reaction in the plants unfavourable to the development of mildew. The resistance induced by the abnormal photoperiods was not, however, permanent, as was shown by the fact that, when the plants were returned to normal daylight periods, they could be infected readily.

Light and the Formation of Perithecia

Volk (68) obtained the best perithecial development on seedlings shaded with two layers of gauze. During the present investigation, it was found that perithecia formed very readily in the summer, and in the fall and spring during periods of clear weather. In December and January, perithecia seldom develop unless the hours of daylight are supplemented by several hours of artificial light.

In the experiments on the effect of light on the development of mildew, which were made in the glass chambers described above, the infected seedlings were kept each time until perithecia had formed. Perithecia began to develop in each chamber at the same time, except on the seedlings that became chlorotic after four days in the dark chamber and died before perithecia became evident. In the chamber shaded with brown paper, perithecia were usually less abundant than in the unshaded one. In general, it appears that perithecia develop best when the days are long and bright.

Light and the Formation of Ascospores

On several occasions perithecia collected in the field were placed either on water or on moist filter papers in two Petri dishes, one of which was wrapped in black paper and the other left unwrapped. In each case, ascospores developed at the same time and equally well in both plates, showing that, in these tests, light had no effect on ascospore development.

Effect of Moisture

Moisture and the Germination of Conidia

Yarwood (71), Brodie and Neufeld (3), and Clayton (5) have shown that conidia of powdery mildews germinate even at zero relative humidity. Clayton found, however, that conidia of *E. graminis Hordei* were much more sensitive than the conidia of *E. Polygoni* to low relative humidity. He did not obtain any germination below 88% relative humidity and, even at 95%, germination was very limited.

In the present investigation, the germination of conidia of *E. graminis* was tested at the three following levels of relative humidity: (*a*) on a dry slide placed on moist filter paper in a Petri dish; (*b*) on a dry slide in a dry Petri dish; and (*c*) on a dry slide placed in an air-tight bottle partially filled with dry barium perchlorate. All the germination tests were made at two temperatures, namely, 6° and 18° C. The test was repeated several times with each variety of *E. graminis*. The results are given in Table XI. The mean percentage of germination at zero relative humidity was lower than at 100%.

TABLE XI

GERMINATION OF CONIDIA OF *E. graminis* AT THREE LEVELS OF RELATIVE HUMIDITY AND TWO DIFFERENT TEMPERATURES, 6° AND 18° C.

Variety of *E. graminis*	Temperature in degrees centigrade	Mean percentage of germination		
		At 100% *R. H.**	At room *R. H.*	At 0% *R. H.* over barium perchlorate
Tritici	6	45.0	26.3	34.6
Tritici	18	35.9	40.2	21.3
Hordei	6	35.4	24.5	19.0
Hordei	18	26.7	9.0	11.4
Avenae	6	47.2	30.0	26.3
Avenae	18	29.7	16.4	17.0
Agropyri	6	49.1	33.6	30.0
Agropyri	18	32.4	19.5	23.1
Mean	6	44.2	28.6	27.5
Mean	18	31.2	21.3	18.2

* *R. H. = relative humidity.*

But, although not shown in the table, germination even as high as 60% was observed at 6° C. and zero humidity. Conidia that germinated at zero humidity and 18° C. were almost all dead within 20 hr., but many of the conidia that germinated at 6° C. showed no shrinkage and evidently remained alive for upwards of 40 hr.

Moisture and Host Infection

Since germination of the conidia of *E. graminis* takes place quite freely at low relative humidity, it would be expected that infection also would proceed normally in a relatively dry atmosphere. Yarwood (71) showed this to be true of *E. Polygoni*, and Honecker (21) noted that persistent drought or too rapid transpiration of the plants favoured cereal mildew infection. Hammarlund (17), however, showed that infection was little influenced by humidity, while, on the other hand, Volk (68) found that high humidity favoured infection and shortened the incubation period of wheat mildew.

At the beginning of the present investigation, inoculated seedlings were incubated in a moist chamber for 24 hours. It was soon found, however, that when the incubated seedlings were not covered by a film of water, better infection resulted. A test to determine the relation of humidity to infection was then made, and included the following treatments: seedlings of wheat and of barley, grown in 4-in. pots, were inoculated and divided into five similar groups, each consisting of two pots of wheat and two of barley. The first two groups were left on a greenhouse bench, one being kept dry so that the seedlings were partially wilted during the first two days following inoculation, while the other was watered normally. A third group was placed on the same bench in a shallow pan of water so as to keep the soil saturated. The last two groups were incubated in a moist chamber for 24 hr. after in-

oculation, the fifth group being sprayed with water at two different times by means of an atomizer. They were then placed on the bench adjacent to the first three groups. The experiment was repeated several times during different periods of the year with similar results.

The results of a representative test are shown in Table XII. In these tests, mildew developed equally well on turgid and on wilted plants. Free moisture on the inoculated surface consistently reduced the percentage of infection, but whether this result was due to the spores having been washed from the plants, or to some harmful effect of moisture on the germ tubes of the conidia, was not determined.

TABLE XII

THE EFFECT OF MOISTURE ON THE INFECTION OF WHEAT AND BARLEY SEEDLINGS BY *E. graminis*

No. of seedlings inoculated	Incubation treatment	Days from inoculation to appearance of conidia	Percentage infection
118	Wilting state for 2 days	5.5	70
123	Normal—on a bench	6	80
121	Pots placed in water	6	80
114	In moist chamber 24 hr.	6	60
127	Sprayed and in moist chamber 24 hr.	6	35

Moisture and the Development of the Disease

There seems to be a great divergence of opinion regarding the effect of moisture on mildew development. Johnston, Fellows, and Melchers (26) reported that powdery mildew occurs only in wet seasons and on lodged grain, while Rivera (2) stated that epidemics of powdery mildew do not occur if the host cells are in a state of maximum turgidity. Butler (4) pointed out that, while one set of conditions may inhibit or reduce the development of a group of diseases, it may favour the spread of another group, and exemplified his statement by the severe epidemic of powdery mildew in England in the dry summer of 1921. Volk (68) has shown that mildew was more virulent on plants growing in soil with 40% water-holding capacity than with 60 to 80%. Similar results were obtained by Yarwood (71) with *E. Polygoni*.

In order to ascertain the effect of moisture on the development of *E. graminis*, seedlings of wheat and of barley grown in 4-in. pots were inoculated and placed in four glass chambers and given the following treatments: (*a*) watered lightly only after the plants wilted; (*b*) watered as usual but the chamber was kept dry; (*c*) watered abundantly, and a pan of water kept in the chamber; (*d*) placed in a shallow pan of water, and the walls of the chamber sprayed frequently with water every day. The treatments were continued for four or five weeks, or until most of the seedlings were dead.

This experiment was repeated, and the average results of both tests are presented in Table XIII. This table shows that, although there was no difference in the percentage of initial infection, the vigour of sporulation and the subsequent spread of infection were better in the dry chambers.

TABLE XIII

THE EFFECT OF SOIL MOISTURE AND RELATIVE HUMIDITY ON THE DEVELOPMENT AND SPREAD OF *E. graminis* ON WHEAT AND BARLEY SEEDLINGS

No. of seedlings in each treatment	Experimental treatment	Per cent of initial infection	Per cent of secondary infection
173	Watered after wilting	83	80
181	Watered normally	85	87
178	Watered abundantly—a pan of water in the chamber	80	70
190	Pots kept in water—walls of chamber sprayed	85	10

In another experiment, 20 pots each of wheat and barley seedlings were inoculated and divided into two equal groups. One group was carefully watered so as not to wet the plants, while, in the other group, the plants were sprinkled with a fine spray of water. The sprinkling was begun when the infections became macroscopically visible. In the first group, a vigorous development and spread of mildew killed most of the seedlings within five weeks. In the second group (sprinkled seedlings), the original infections turned into mycelial mats with very little sporulation, thus resulting in limited subsequent spread. This experiment was repeated twice, with similar results.

Mention here may be made of the fact that it is a common experience in tests for varietal resistance to find that seedlings watered by sprinkling water over the plants usually appear resistant to mildew.

Moisture and the Formation of Perithecia

In the experiments on the effect of moisture on the development of mildew, the infected seedlings were kept for four to five weeks until perithecia developed. No difference was apparent in the development of perithecia under different moisture conditions. Even on the plants that were sprinkled with water, perithecia developed in the pustules on the underside of the leaves or on parts otherwise protected from the daily washing by water.

Moisture and the Formation of Ascospores

Graf-Marin (16) obtained ascospores when perithecia were either soaked in water or kept in a moist chamber at 90% relative humidity. Similar results were obtained by the author with perithecia collected in the field. In the greenhouse, however, seedlings bearing abundant perithecia were many

times kept for several weeks, until the infected parts shrivelled and decayed (on the damp soil), but ascospores were never found in the perithecia. Neither did ascospores form in perithecia collected in the greenhouse and immediately placed on water or on moist filter paper in a Petri dish. But, when such perithecia were allowed to dry and were then moistened again, ascospores developed quite readily. The drying and wetting method has been tested many times on perithecia collected in the greenhouse, and ascospores were obtained every time, indicating that alternate drying and wetting of the perithecia of *E. graminis* is necessary to induce the development of ascospores.

Effect of Organic and Inorganic Materials

Plant Extracts and Germination of Conidia

Graf-Marin (16) obtained 3% germination of conidia in water, 2% in extract of diseased leaves, and 23% in extract of healthy leaves. He concluded that the higher germination in the extract of healthy leaves was due to the presence of the elements that the fungus needs for its nourishment, and the inhibitory effect by the extract of diseased leaves was due to the presence of staling products. His results would indicate that the food elements had a chemical stimulation upon the germination of the conidia. If mildew conidia respond to chemical stimuli, it might be expected that extracts of susceptible and resistant hosts would show a differential stimulation.

A number of germination tests were, therefore, made in which both susceptible and immune host extracts and also pieces of host tissue were used. Conidia of both barley and wheat mildew were dusted on to slides that received the following treatments: (*a*) extract of a susceptible host deposited in small drops; (*b*) extract of an immune host deposited in small drops; (*c*) small pieces of susceptible host leaves placed on slides; (*d*) small piece of immune host leaves placed on slides; (*e*) small drops of distilled water, as a check. The slides were kept in moist chambers at approximately 20° C. Usually 24 hr. elapsed before the counts were made. As usual, the results varied from one test to another, a fact that may explain certain conclusions based on single tests. The mean percentage of germination, as shown in Table XIV, does not indicate any stimulation of conidial germination by the susceptible host extract. Neither was there any adverse effect upon germination by the extract of an immune host.

Careful observations of the germinated conidia in close proximity to the drops of extracts or bits of plant tissue revealed that even conidia that appeared to be in contact with the drops or the host tissue germinated in all directions, indicating that there was no chemotropic response.

Fertilizers and Chemicals and Host Susceptibility

Considerable attention in recent years has been devoted by many investigators to the effect of various fertilizers and chemicals upon the development of plant diseases, including powdery mildew. Spinks (59) tried various proportions of nitrogen, phosphate, and potassium fertilizers both in nutrient solution and soil culture, and found that nitrogen increased susceptibility

TABLE XIV

THE EFFECT OF HOST EXTRACTS ON THE GERMINATION OF CONIDIA OF *E. graminis* AT 20° C. AND 100% RELATIVE HUMIDITY

Medium	Mean percentage germination	
	Spores on surface of extract	Spores on dry slide between drops
Susceptible host extract	47	51
Resistant host extract	49	48
Distilled water	42	52
	Spores in near vicinity of host tissue	
Small bits of susceptible host	53	54
Small bits of resistant host	41	50

while phosphate and especially potassium increased resistance in wheat and barley to mildew. His results were, in general, confirmed by other investigators (31, 64). Schaffnit and Volk (55) found, however, that an excess of potassium or phosphorus increased the resistance in cereal plants only subsequent to the main vegetative period. In the early stages of growth, no difference in susceptibility between normally and excessively nourished plants was observed.

Other chemicals, such as silicon (14), lithium (27, 59), cadmium (2), manganese (6), copper (44), and boron (11) have also been reported to increase resistance in plants to powdery mildew. Some of these reports are, however, not in accord. For example, Eaton (11) observed that barley grown in a quartz-sand bed supplied with boron was not attacked by mildew, while Yarwood (73) obtained no noticeable effect on the susceptibility to mildew of oats grown in nutrient solution to which boron was added. Olsen (44) reported that barley grown in nutrient solution containing traces of copper was not attacked by mildew, whereas Salmon (51) could not get any control of barley mildew by the addition of copper to the culture solution.

In the experiments carried out in Winnipeg during the last two years, the effect of various fertilizers and chemicals on the development of mildew was tested. For these experiments, seedlings of susceptible varieties of wheat and barley were grown in two different soils, medium heavy loam and ordinary unwashed sand, to which various chemicals individually as well as in different combinations were applied in dry form at the time of planting, or in solution once a week for the duration of the experiment. All chemicals tested were applied at two different rates. Seedlings were inoculated usually when the first leaf was fully opened, and observations were made at least until infection had spread to the second leaf. The chemicals used and the rates of application are shown in Table XV.

TABLE XV

THE EFFECT OF VARIOUS FERTILIZERS AND OTHER CHEMICALS ON THE SUSCEPTIBILITY OF WHEAT AND BARLEY SEEDLINGS TO *E. graminis*

Chemicals	Rate of application*, lb./acre	Infection type		Development of perithecia
		Wheat	Barley	
N_2, as $Ca(NO_3)_2$	100, 200	All type (4) infections	All type (4) infections	Abundant
$(NH_4)_2SO_4$	100, 200			Abundant
P_4, as H_3PO_4	100, 300			Few
NH_4—superphosphate	100, 300			Few
K, as K_2SO_4	100, 200			Few
N P K	200, 400			Common
B, as Borax	10, 50			Common
Cu, as $CuSO_4$	10, 50			Few
Mn, as $MnSO_4$	10, 50**			Few
Li, as LiCl	10, 50**			Few
Mo, as MoS_2	10, 50			Few
B + Mn	Both light and heavy applications used***			Common
B + Mn + Cu + Li	Both light and heavy applications used***			Few
N P K + B	Both light and heavy applications used***			Abundant
N P K + Mn	Both light and heavy applications used***			Common
N P K + Cu	Both light and heavy applications used***			Common
K + B	Both light and heavy applications used***			Few
K + B + Mn + Cu	Both light and heavy applications used***			Few
N P K + B + Mn + Cu + Li	Both light and heavy applications used***			Few
Cd, as $CdCl_2$	50 cc. of M/7000 per pot once or twice per week			None (Test made in (winter)

** Rate of application is based on the weight of a particular compound per acre of surface area.*

*** The heavier rates of applications of lithium and manganese were somewhat toxic to the seedlings.*

**** Same two rates as indicated for each respective chemical, for example, in the combination of B + Mn, 10 lb. of borax and 10 of $MnSO_4$ were used for light application, while 50 lb. of each were used for heavy application.*

Since the various chemicals were not all tested at the same time, the percentages of infection are not strictly comparable and, therefore, are not included in the table. In each test, however, untreated seedlings were included as a check on the effect of the treatments. Under the conditions of these experiments, none of the chemicals so far tested produced any effect on the susceptibility of the host to mildew.

The Effect of Chemicals on the Development of Perithecia

In the experiments with chemicals described above, observations were also made on the development of perithecia. As may be seen in Table XV, perithecia developed more abundantly on the seedlings with rank growth induced by heavy applications of the nitrogenous fertilizers, a result in disagreement with those of several investigators (9, 12, 16, 20, 28), who found that perithecia are formed only on old or exhausted plant parts. There was some evidence that the formation of perithecia was stimulated by the addition of boron, but no special test was made to determine this point.

Discussion

With the increased interest in the occurrence of powdery mildew on cereals during the last few years in Canada, certain problems in the biology of the pathogen (*E. graminis*) have been encountered which seemed to require further elucidation. For example, the common belief that mildew occurs only in wet summers or that the mildew organism overwinters in the perithecial stage, were not in accord with the results of certain investigations (15, 21, 45, 68, 71). Other problems, such as the conditions conducive to the formation of perithecia and the effect of temperature on the development of the disease, were either not well understood or had not received much attention in the past.

The present investigations by the author in Manitoba have confirmed the results of others (21, 68) that the development of powdery mildew on cereals is favoured by relatively dry weather. In 1941, when powdery mildew on wheat and barley reached epidemic proportions in that province, the early summer was dry and relatively hot; but, in 1942, in spite of the wet summer, only scattered local infections occurred, and these only developed late in July. Greenhouse experiments have shown quite conclusively that, not only do conidia germinate in a dry atmosphere, but infection and mildew development on wheat and barley proceed normally in low relative humidity. Although in *E. graminis* no difference was observed in the amount of infection and vigour of sporulation on wilted and turgid plants, development of the disease was appreciably checked at approximately 100% relative humidity. These results are in accord with those obtained by others (21, 68, 71). Moreover, the sprinkling of infected plants with water definitely checked the development of mildew. Whether the effect was only physical—the water having washed off the conidia and damaged the mycelium—or was there some physiological reaction involved, has not been determined. Most of the rain in the Prairie Provinces during the summer comes in short heavy showers, and, no doubt, such showers have a considerable effect in checking the development and spread of powdery mildew.

The experiments on the effect of environment upon mildew development indicated that the effect of light is only indirect, acting through the host by either determining the availability or probably by changing the composition of food. This relation would seem to account satisfactorily for the indifferent

mildew development under adverse light conditions. High daylight intensity and medium to long days are conducive to high ascorbic acid content in cowpea plants (49). Possibly light may also influence ascorbic acid production in cereal plants. Since it has been observed (47) that vitamin B_1 (thiamin) is beneficial to powdery mildew, it may be that in poor light or during very short days, a deficiency of vitamin C (ascorbic acid) or some such compound limits the development of mildew. Or, possibly, plants under adverse light conditions do not provide sufficient carbohydrates for the development of mildew, as suggested by Trelease and Trelease (65). Under natural conditions in the Prairie Provinces, during the summer months there is ample light to promote normal plant development, and hence light can hardly be a limiting factor in this area.

In previous investigations on *E. graminis*, temperature, another important environmental factor, has not received much attention. Normal summer temperatures do not seem to have very conspicuous effect upon mildew development in Manitoba, but, in greenhouse experiments, a continuous temperature of from 25° to 27° C. prevented further spread of mildew from initial infections. Alternating temperatures, on the other hand, induced the development of perithecia, thus making individual pustules rather short-lived. These results indicate that temperature is an important factor in mildew epiphytotics. Better development of mildew in shade than in direct sunlight (65, 68) might have been due to differences in temperature rather than in light intensity, as the temperature was probably considerably lower in the shade. Furthermore, the prevalence of local infections of powdery mildew in the park-belt area of the Prairie Provinces, might be explained by the fact that, owing to the undulating topography, local differences in air temperature are commonly present. The greater prevalence of the disease in low areas is probably, therefore, attributable to the colder air rather than to greater humidity.

Another debatable point in the biology of *E. graminis* is the stage in which the organism overwinters. Perithecia of *E. graminis* have been shown in the present investigation to be rather resistant to sudden changes in temperature, and, when kept dry, to remain viable for as long as two years. From this fact it might be inferred that mildew perithecia on stubble and scattered bits of straw in the field would be able to survive a winter even with comparatively little snow. Nevertheless, mildew on cereals has never been a problem in the flat treeless plain of southern Saskatchewan, where, owing to lack of snow cover, volunteer or winter grain plants seldom survive the winter. Moreover, perithecia of *E. graminis* in Manitoba have been observed to produce and expel their ascospores in the fall. The fact that alternating temperatures (e.g., between 10° and 30° C.) induced perithecia to develop on young seedlings as early as 13 days after inoculation, makes it evident that their development is not dependent on the exhaustion of food in the host, as has been

claimed by some investigators (9, 12, 16). Furthermore, perithecia of *E. graminis* on dead host tissue do not undergo a period of dormancy as do, for example, the teliospores of cereal rusts. If the perithecia serve as the overwintering stage, these facts would certainly indicate a very poor adaptation on the part of the organism. If, on the other hand, the organism overwinters as mycelial infections or mats, as the present investigation indicates, the failure of the disease to establish itself or persist in the treeless plain of the Prairie Provinces and its ability to do so in the parts of Canada where winter grains are more successfully grown, become readily comprehensible.

The question then arises as to what is the main function of the perithecia. In the Prairie Provinces at least, their function seems to be to tide the fungus over adverse periods during the summer. In these provinces, there is usually a hot period during the latter part of July and early August. At this time, the lower leaves and sheaths of the common cereal crops are dead. Mildew ordinarily does not develop on the uppermost leaves, apparently owing to the higher temperature of the leaf surfaces exposed to the sun. There is, therefore, a period of several weeks with relatively little or no host metabolism, that is to say, with little or no vegetative growth on which the pathogen can continue to develop. As shown in the present investigation, conidia of *E. graminis* do not survive for any length of time at temperatures above the freezing point. Moreover, in Manitoba, abundant development of perithecia in the field has been observed early in June, but perithecia containing ascospores have never been observed on living host tissues. As soon, however, as the infected tissue dies and the perithecia are alternately wetted and dried, the formation of ascospores proceeds rapidly. Fall rains, which commonly occur in the Prairie Provinces, favour the growth of volunteer plants, as well as the development and expulsion of ascospores. It would, therefore, appear more logical to regard the perithecial stage as an extremely fine adaptation to carry the organism over hot periods in the late summer rather than as an adaptation for overwintering. A further adaptation is seen in the fact that ascospore production is stimulated as a result of wetting and drying, an adaptation well suited to fall conditions when new growth is available for infection.

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